

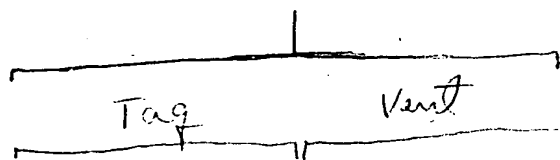
DU.GEL

- 01/19/95 - 09:20 pm

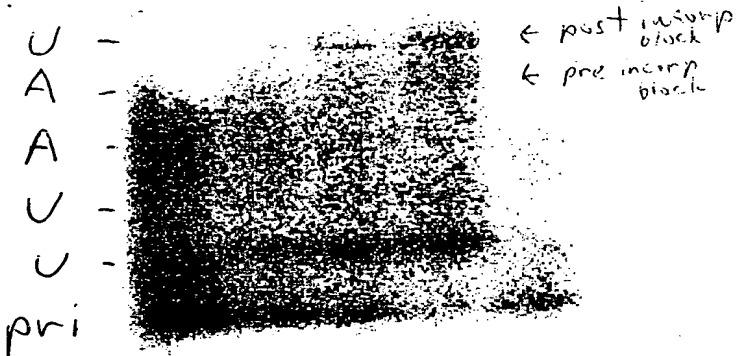
2.00x Counts

29.99  200.0

- J ATP <sup>mut</sup> (T) primer



min 0 2 5 10 0 2 5 10



← primer degraded

Witnessed & Underscribed by me,		Date	Invented by	Date	T P
			Recorded by		

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Miniprep for Azouls  
PLAC PCR

114

From Page N \_\_\_\_\_

miniprep #

# 1-20

21-40

41-60

61-80

81-100

101-120

121

Tag

+

+

+

+

+

+

Blue colony

Tag + Deep. Val

W/N

0

.05

0.1

0

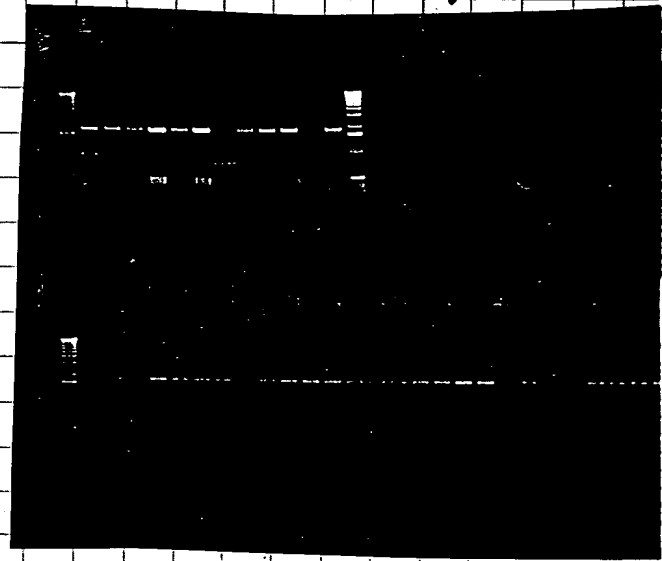
.05

0.1

grow 1/10 30°C, 2 ml circle grow + 100 µg/ml Amp

miniprep same as p41, 4 using 1 ml cells

digest as per P 93 A1F IV Act II, Eco RI 5 µl ~~10 µl~~ miniprep  
1-40 on 40 well comb, load 10 µl  
conclude resolution not good  
enough for ~500 bp range  
1-40 on 30 tooth comb, load 2  
need more DNA in digest and load 2



T Pag N

Witnessed & Underst od by me,

Deanna Polansky

Date

2/16/95

Invented by

Record d by

Dat

127-55  
3075

Repeat digest of P114 for fidelity  
assay: use 10  $\mu$ l miniprep

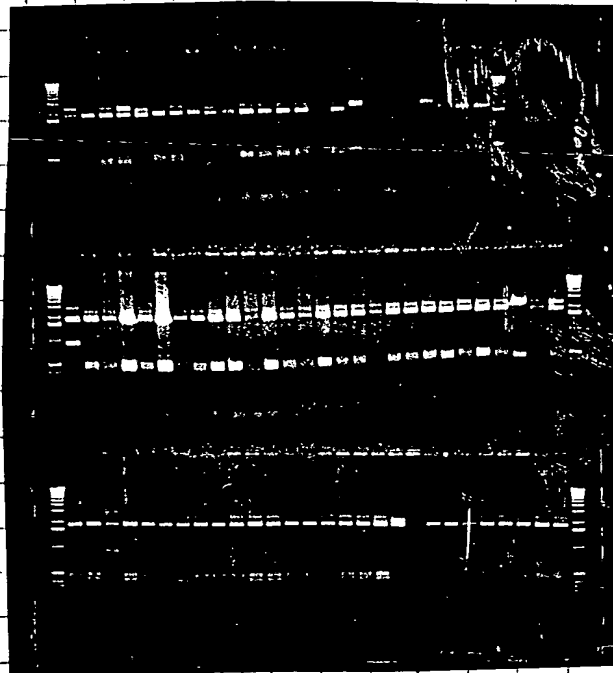
ag N

digest 2h 37°C load 20  $\mu$ l

NEB buffer	2 $\mu$ l	✓
1 $\mu$ l Afl III	0.3	
1 $\mu$ l Aat II	0.1	
1 $\mu$ l Eco RI	0.5	✓
water H <sub>2</sub> O	7.1	✓

VP = 10  $\mu$ l

digest 10  $\mu$ l miniprep  
VP = 20  $\mu$ l



miniprep

5-7-8

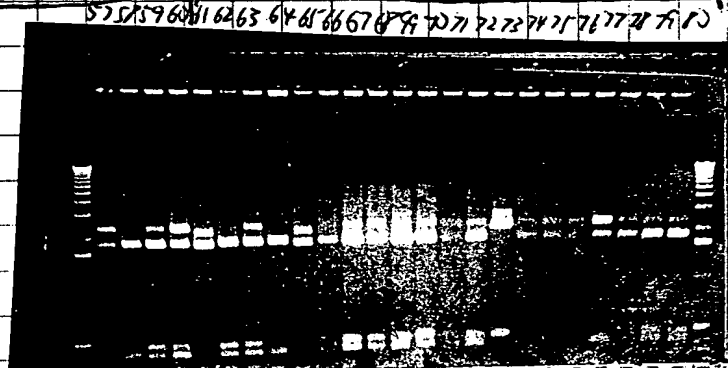
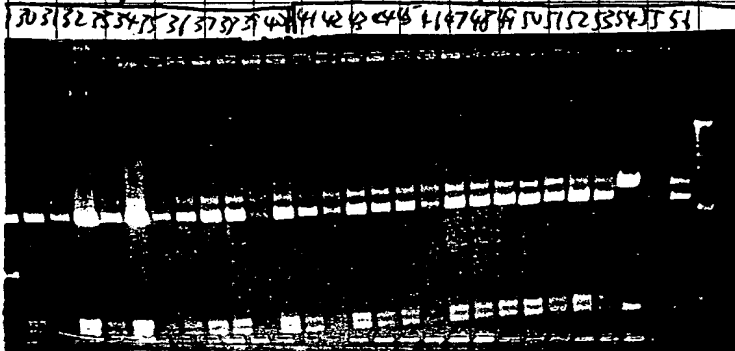
29-56

1-28

0.5 mM Mn

1 0.1 mM Mn

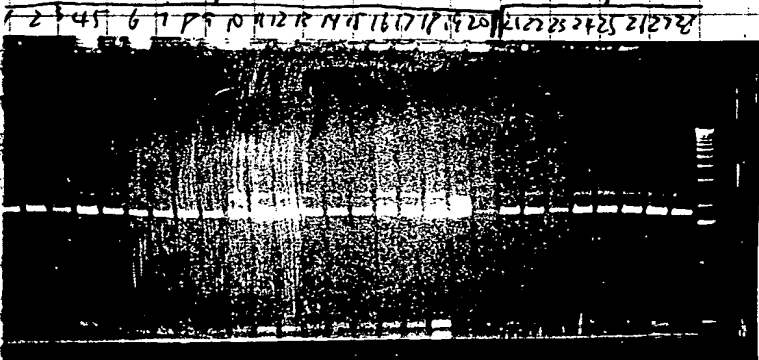
no Mn, + Vent



16  
2  
11  
4  
4

no Mn

0.5 mM Mn



Del ↑ ? ↑ ?

full length  
for #13  
rel P123

To Page No. \_\_\_\_\_

Read & Understood by me, ..

*Carla Boland*

Date

2/16/95

Invented by

*[Signature]*

Date

1-30-95

Recorded by

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

116

From Page No. \_\_\_\_\_

	full length	+ 410 bp (no Ant II)	+ 465 bp (no Ant III)	(7)	(*)	(F)
#1-20, Tag 0 Mn	17 19	(Q22 P 123 SST I)		1	X	✓
21-40, Tag 0.05 mM Mn	18 20				X	✓
41-60, Tag 0.1 mM Mn	18 2					
61-80, Tag 0 Mn + D Vent	12 17	X	1	2		4
81-100, Tag 0.05 mM Mn + D Vent	18 2					
101-120, Tag 0.1 mM Mn + D. Vent	16 4					

See well  
Table on P 124  
after Dr. I and SST  
cuts

- ⊗ 900mer lacks RI site in MCS  
⊕ no result, i.e. not enough DNA  
to be sure about cut.

0.1 mM Mn, Tag + Deep Vent

109 120

confirmed deletions  
miniprep #19, 61, 65

only 410 or 465 removed  
so it's 2.2 bp

1.8 bp  
has 410 and 465 removed

miniprep #



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Date

Investigated by

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Deena Polansky

2/16/95

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1-31-95



Page N

### Still Needed 8

cut with  $\text{Ora I}$  to see if full length  $\text{lac Z}$  is present (assuming either  $\text{Aa I III}$  or  $\text{Aa I II}$  recognition region had a point mutation generator). There are the "410" and "465" bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 120

plus  $\text{Aa I II}$ ,  $\text{Aa I III}$

cut with  $\text{Sst I}$  to see if  $\text{R1}$  site in MCS was a point mutation (or very small deletion (see on P. 107 at bottom) resulting in the "90mers")

miniprep # 3, 29

Recut with  $\text{17 } \mu\text{l}$  miniprep and load  $30 \mu\text{l}$ ?

<sup>2.5  $\mu\text{l}$  reaction</sup>  
Trying to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. \_\_\_\_\_

Read & Understood by me,

Erica Boland

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

From Page No. \_\_\_\_\_

SAP	CPM1	TIME
A1	1 4976.00	0.50
	2 5216.00	0.50
	3 4500.00	0.50
	4 16920.00	0.50
A6	5 17020.00	0.50
	6 16156.00	0.50
	7 3926.00	0.50
A1	8 3822.00	0.50
	9 4054.00	0.50
	10 15974.00	0.50
A2	11 16520.00	0.50
	12 15478.00	0.50
	13 4684.00	0.50
A3	14 4752.00	0.50
	15 4606.00	0.50
	16 17622.00	0.50
A0	17 16806.00	0.50
	18 17742.00	0.50
	19 4186.00	0.50
CTI	20 3966.00	0.50
	21 3986.00	0.50
	22 14842.00	0.50
LTI	23 14704.00	0.50
	24 15620.00	0.50
	25 4458.00	0.50
L	26 4644.00	0.50
	27 3970.00	0.50
	28 16730.00	0.50
L	29 16914.00	0.50
	30 15684.00	0.50
	31 4864.00	0.50
L3	32 5020.00	0.50
	33 4538.00	0.50
	34 15236.00	0.50
L18	35 17922.00	0.50
	36 17898.00	0.50
	37 12.00	0.50
Aquasol	38 16.00	0.50
blank	39 16.00	0.50

delivered 10  $\mu$ l with p10 (wiped tip)  
rinse 3x into 4 ml aquasol

each dilution had 3  $\mu$ l of 1  $\mu$ C/ml 3H TTP

To Page 1

Witnessed &amp; Understood by me,

Date

Invented by

Date

Record d by

Deena Boland

2/16/95

RJP

1/25/95

New rTag dilutions

Proj ct N . \_\_\_\_\_

Bo k No. \_\_\_\_\_

119

ag N . \_\_\_\_\_

#

EKB T1

77.4

18.6  $\mu$ l

323 units/ $\mu$ l (P91)

Tag dilution buffer

4922.6  $\mu$ l

1981.4  $\mu$ l

$V_f = 5$  ml

(5 units/ $\mu$ l)

$V_f = 2$  ml

(3 units/ $\mu$ l)

both are labelled "1-31-95 rTag"

To Pag No. \_\_\_\_\_

ss d & Und rstood by m ,

Date

Inv nted by

Date

Deena Golap

2/16/98

R cord d by

1-31-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Accuracy of delivering 1  $\mu$ l  
with P2 pipetman for Tag storage

From Page No. \_\_\_\_\_

add 1  $\mu$ l, 10 times to a weigh boat with  
a drop of H<sub>2</sub>O in it so tip can be rinsed  
several times. Use storage buffer at 0°C (on ice)

add H<sub>2</sub>O Total 0.0000

1  $\mu$ l  
2

3

4

5

6

7

8

9

10

0.0119  $(\frac{94}{103}) = 0.011$

note 10  $\mu$ l SB = 0.01  
.0094

So ~ 1.1  $\mu$ l was added instead of the 1  $\mu$ l intended

conclude 2  $\mu$ l is better to add for units

stock	for Tag unit assay	use	CP in unit assay	for 6607 Rxs
3 $\mu$ l	0.5 M TAPS pH 9.3	150 $\mu$ l	25 mM $\rightarrow$	✓ X 206
120 $\mu$ l	1 M MgCl <sub>2</sub>	6 $\mu$ l	2 mM ✓	0.1 M DTT ✓ X 3
1 $\mu$ l	3 M KCl	50 $\mu$ l	50 mM ✓	10 mM DTPA ✓ X 6
				2 $\mu$ l 12 mM / ml Salicin ✓ X 8340
				Tarbo Chloroform / GNA ✓ X 12
				H <sub>2</sub> O ✓ X 2293
				CP = 3

use 230  $\mu$ l / 2  $\mu$ l Tag unit assay mix

Witnessed & Understood by me,

Date

Invented by

Date

2/16/95

Recorded by

2-1-95

Page No.

**B ok No.**\_\_\_\_\_

Continued from P 111

SSI and DSI costs of materials

age N	min at 1 hr										post control										control		
	↓	(1)	1	2	3	4	5	6	7	8	9	10	(11)	12	13	(14)	15	16	17	17	18	20	(21)
up #		30	54	58	64	73	87	92	103	108	113	120	30	3	29	30	20	39	71	74	75	76	31
	10	10	—————→										10	—————→		25.6	—————→						
buffer 4	2	2	—————→										2	—————→		3	—————→						
I	1	1	—————→																				

$\sqrt{III}$	$\sqrt{0,3} \rightarrow 0,45$	$\rightarrow$	$\checkmark$
$+ II$	$\sqrt{0,1} \rightarrow 0,15$	$\rightarrow$	$\checkmark$
$+ I$	$10,5 \rightarrow$		
$- R$	$0,75$	$\rightarrow$	$\checkmark$

[illegible]

37°C 2 hr

was only a  $\frac{1}{2}$  cent by 1 hr  
add 0.5 ml more ~~say~~

for  $DrdI$

<u>mini prep</u> <u>#</u>	<u>6p</u> <u>Progenies</u>
1	1
2	1
3	1
4	1
5	1
6	1
7	1
8	1
9	1
10	1
11	1
12	1
13	1
14	1
15	1
16	1
17	1
18	1
19	1
20	1
21	1
22	1
23	1
24	1
25	1
26	1
27	1
28	1
29	1
30	1
31	1
32	1
33	1
34	1
35	1
36	1
37	1
38	1
39	1
40	1
41	1
42	1
43	1
44	1
45	1
46	1
47	1
48	1
49	1
50	1
51	1
52	1
53	1
54	1
55	1
56	1
57	1
58	1
59	1
60	1
61	1
62	1
63	1
64	1
65	1
66	1
67	1
68	1
69	1
70	1
71	1
72	1
73	1
74	1
75	1
76	1
77	1
78	1
79	1
80	1
81	1
82	1
83	1
84	1
85	1
86	1
87	1
88	1
89	1
90	1
91	1
92	1
93	1
94	1
95	1
96	1
97	1
98	1
99	1
100	1

2677 — Dr J

## Results

control	2.7kb (insert)	1.8	0.8	full length expression
#73	"	"	"	"

58,64
87,120

54, 78, 103  
113

么才

Full Length  
6:00 pre-est

maniprip  
5 and 27  
have full  
length. We  
based on presence  
of 410, 465 bp  
30 R1 was probably  
small or poor  
mutation

all no  
results  
of P.I.  
all full  
finger  
here based  
on pictures  
of 410 46566  
w/ain more OK  
not here

↑  
mini  
#75  
has no b.p.  
full brother  
low is  
present

**To Page No.**

ised & Understood by m

serena Polansky

## Dat

2/16/95

Invented by \_\_\_\_\_

**Recorded by**

**Dat**

225

**From Page No.\_\_\_\_**

Full length  
line

percent rearrangement

moniprep #

 $M_n$  (mM)

Deep Vent

1-20

0

19

50%

21-40

05

20

41-60

0.1

18

 $10^6$ 

61-80

0

17

157

81-100

05

+

18

105

101-120

0.1

+

7

2076

**T Pag M**

**Witness d & Understo d by me,**

**Date**

**Invented by**

## Dat

Deena Polay

$$2 \mid 16 \mid 95$$

**Recorded by**

containing 1 unit of Tag

age No.		Run#	at 100% added to reaction	Tag unit added mix	put 3 ul H <sub>2</sub> O / 4 mix 10 1X
1. experiments					
J. Soler of 1-20-95					
2x R26 0.1% TN (Turner/NP40)	1-3	2	(.0004%)	48 ul	= 48 ul mix
0.2% BT Brj	4-6				20+2 X Enrg
0.2% TX Triton	7-9				gives 1/2 = 50
0.01% TN	10-12		(.0004%)		
0.02% BT	13-15				
0.02% TX	16-18				
1.0% TN	19-21		(.04%)		
2.0 BT Brj	22-24				
2.0 TX	25-27				
No detergent	28-30				
(1.1X)	31-33	3.64			
(5X) → dilute 1/2.5 = .04%	34-36	2			
2x R26 0.1% + Enrg	37-39	1			
2x TFI 0.1%	40-42				
2x Vent buffer	43-45				
5 ul dil = 0.04% / ul	46-50	2			

Reaction on 10  
this page 2-3-95  
152 3-9-95  
167 4-4-95  
36, 10 5-26-95  
52, 10 5-27

10' 74°C, 10' 100%  
spot 40 ul on 6 FC  
solution used 100%  
new stocks  
dil buffer 0.9-20-94 (P55, 7)

Test Run mix	Mix P.W	
3 EKB 15% / ul 1-31-95		
no dil	2	48 ul
1/125	2	
EP9407	2	
1/125	2	

made new mix with stock shown in  
red on P120 and repeated experiment  
on 2-3-95 - results on next page (P122)

incorporation!

added 5 ul of #12 (5X) into 12 ul Tag dil buffer P55, 7

Issued &amp; Understood by me,

Sarah A. Bolamp

Date

2/10/95

Invented by

Recorded by

Date

 2-1-95  
2-3-95

To Page No.

# BEST AVAILABLE COPY

122

Project No. \_\_\_\_\_

Book No. Avp

TITLE unit/pt

Relative  
to Tray

Tray = 5

u/l N

From Pr

1E	1	8410.00	}	8819	.037		.03
	2	9136.00					
	3	8912.00					
	4	7465.00	}	7552	.033		.03
2E	5	8664.00					
	6	7728.00					
	7	7737.00	}	7580	.032		.03
3E	8	7235.00					
	9	7769.00					
	10	7579.00	}	6778	.029	✓	.02
4E	11	(3001)00					
	12	6178.00					
	13	7484.00	}	7812	.033		.03
5E	14	7833.00					
	15	8119.00					
	16	6228.00	}	6566	.027	✓	.00
6E	17	6715.00					
	18	6755.00					
	19	8215.00	}	7824	.033		.03
7E	20	8743.00					
	21	6514.00					
	22	7996.00	}	8413	.035		.03
8E	23	8661.00					
	24	8581.00					
	25	7644.00	}	7533	.031		.03
9E	26	6981.00					
	27	7976.00					
	28	4900.00	}	4989	.021	} no detector looks low can try + detector in unit assay	.00
10E	29	4647.00					
	30	5419.00					
	31	7509.00	}	7702	.032		.03
11E	32	6923.00					
	33	8674.00					
	34	8196.00	}	2075	.034		.03
12E	35	7970.00					
	36	8060.00					
	37	8015.00	}	7442	.031		.03
13E	38	7358.00					
	39	6954.00					
	40	8055.00	}	8479	.035		.035
14E	41	8359.00					
	42	9023.00					
	43	7844.00	}	7611	.032		.03
15E	44	7351.00					
	45	7638.00					
	46	9312.00	}	9580	(0.04)		.00
	47	9496.00					
	48	9290.00					
avg	49	9726.00	}				by definition
25	50	10073.00					
lat	51	58661.00					
	52	60427.00					

ave = 5754.4  $\Rightarrow$  1,478,600 cpm/50  $\lambda$  Rxn  
37.2 cpm/pmol

To Page No

Witnessed & Understood by me,  
Deanna Polanco

Date  
2/16/95

Invented by

Recorded by

Date

2-3-95



32 P 23mer degradation reaction complete

[illegible][illegible]

age N .

1/5 Vent dil buffer

1/5 Vent dil buffer

Vent dil buffer

dilute 1/5  
with 10mm  
Tris HEPH 7.5

CF

50 mM Tris HEPH 7.4

1 mM DTT

0.1% NP40, Tween 20 each

50% glycerol

100 mM KCl

20 mM

0.2 mM

0.02

10%

10 mM

run 16% PAGE plus new reactions on 2-13-95

16% PAGE see P 144, 1

# 24 25 26 27 28 29 30 31 32 33

Vent buffer

2

2

2

1

#1 P125

Vent buffer

4

4

✓

heavy PPO-83

4

4

4

✓

20 mM

2

2

2

✓

CF 2 mM

x #10 P125

4

4

✓

Cit has MgOAcinit

Cheng mix

w/ P126

0.6

-

-

✓

P19 (P126 .077 pmol circles /  $\mu$ l)

5

5

✓

.038 pmol circles

0.01  $\mu$ l

2

2

2

2

2

2

2

total

0.1  $\mu$ l

2

2

2

2

2

2

2

glycerol

2.8

2.8

✓

15.4

15.4

13.4

13.4

8.6

8.6

13.4

13.4

11

4.2

✓

my Cheng has on

1% glycerol at 1X

need 7% more

4% = 20  $\mu$ l

40% Acrylamide

200g

0.8% Bis

4g

H<sub>2</sub>O

7% PAGE

start 1700 V at 1:45 pm

at 2 P Watts, 15 mAmp

- my Brad

get ~ 2.4 cm/hr

or need 3 hr

500 ml

run 1-10

11-20 pri

21, 22

24-32 pri

10 empty

big plate

went to 500 with

constant get

2000-2200 ✓

8.7 cm/hr

To Page No.

read &amp; Understood by me,

Sandra Polak

Date

2/16/95

Invented by

Recorded by

Date

2-10-95

2-13-95

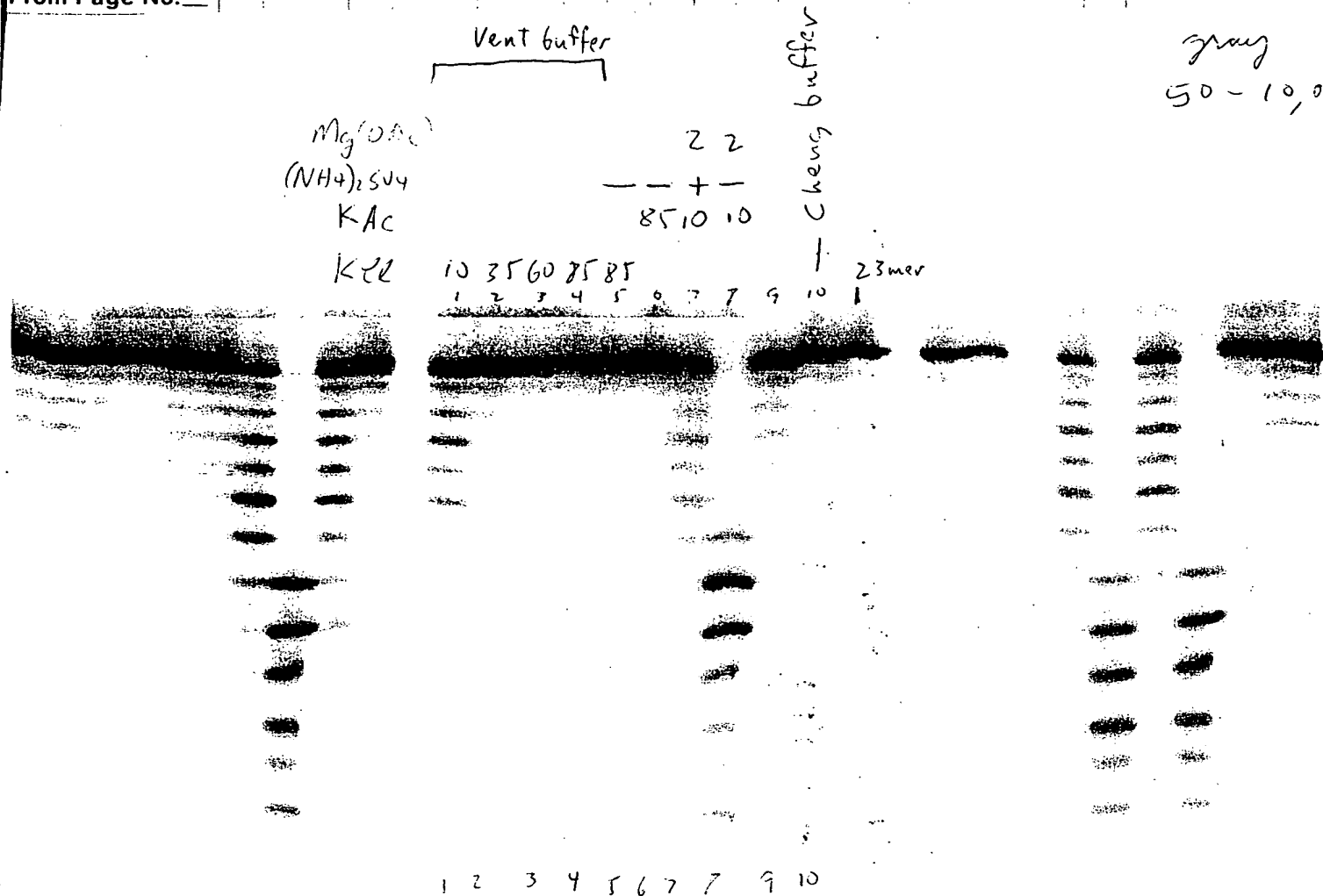
Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

128

From Page No. \_\_\_\_\_



Results:

- #1, 10 KCl +  $MgSO_4$  is same as KAc,  $MgOAc$  - get degradation if  $K \leq 1$
- 1-5 increasing ionic strength eliminates degradation. #5 also 85 mM KAc same as KCl 85 mM
- 8 leave out  $(NH_4)_2SO_4$  get best result degradation of all (don't have (-) $(NH_4)_2SO_4$  for 10 mM KCl and  $MgSO_4$  only 85 mM this result also consistent with ionic strength effect
- 9 substitute Tricine for Tris in Vent buffer has no effect
- 10 complete Cheng buffer - no degradation can be fully explained as due to 85 mM KAc - see # 4, 5 - 85 mM KCl or KAc & degradation in Vent buffer

Witnessed & Understood by m ,

Dat

2/16/95

Invent d by

R c rd d by

Dat

2-13-95

T Pag

- 02/14/95 - 06:45 pm

1.00x Counts

49.97



10000.00

D

Vent  
buffer

Cheng

NEB

RL

5x  
G-TM

RL

Vent (NEB) 23-mp/19

Cheng G-TM 23-mp/19

23 mer

.02 .2 .02 .2 .02 .2 .02 .2 .2 .2

:Units  
Vent

23 mer is strongly  
protected when annealed  
to m13 ssDNA

To Page No. \_\_\_\_\_

ss d &amp; Understood by me,

Date

Invented by

Dat

Zachary Polansky

2/16/95

Record d by

130

Tne vs T<sub>0</sub>g Project No. \_\_\_\_\_  
 Book No. \_\_\_\_\_

TITLE

effect of K<sub>2</sub>ell on pol on M13  
 and primer degradation

From Page No. \_\_\_\_\_

(1) (2) (3) (4) (5) (6)  
 50 75 100 150 75 100

10 X Tag PCR buffer	8 $\mu$ l	→	✓	✓
K <sub>2</sub> ell 0.5 M	4	8	4	8
<sup>32</sup> P 23. m p19 (0.064 pmol <sup>23. m p19</sup> / $\lambda$ )	8 $\mu$ l	→	✓	✓
10 mM 4dNTPs	1.6 $\mu$ l	→	✓	✓
Mg <sub>2</sub> 50 mM	2.4 $\mu$ l	→	✓	✓
T <sub>0</sub> g 0.4 $\mu$ / $\lambda$	2	2	2	2
Tne 0.8 $\mu$ / $\lambda$				
H <sub>2</sub> O	58	54	50	58
	54	50	54	50

preheat tubes to 70°C, start with 2  $\mu$ l pol

VP = 80  $\mu$ l

remove 10  $\mu$ l at 1, 2, 5, 10 min to 5  $\mu$ l cycle seq stop

\* rTag EKBT1 1-31-95 5  $\mu$ /  $\lambda$  } both diluted in Tag dil buffer  
 Tne 5  $\mu$ /  $\lambda$  A. Goldstein

<sup>32</sup>P 23 mer same as P.75 (0.267 pmol 23 mer /  $\lambda$ )

<sup>32</sup>P 23. m p19

<sup>32</sup>P<sub>primer</sub> 0.267 pmol 23 mer /  $\lambda$  15.8  $\mu$ l 14.2 pmol 23 mer tr

M13 m p19 0.2  $\mu$ g /  $\lambda$  50  $\mu$ l (4.2 pmol circle  
 (0.084 pmol circle /  $\lambda$ )

1 mT<sub>0</sub>s 7.5 0.6 6.6  $\mu$ l 0.064 pmol <sup>23. m</sup>

use 1  $\mu$ l / 10  $\mu$ l re

To Pag 1

With ssed & Understood by me,

Date

Invented by

Dat

Deena a Goldstein

2/16/95

Record d by

2-15-95

25-27 28-30 31-33 34-36 37-39 40

Tag N	(7)	(8)	(9)	(10)	(11)	(12)	
vent	8 <sup>th</sup>	8 <sup>th</sup>	8 <sup>th</sup>	8 <sup>th</sup>	8 <sup>th</sup>	8 <sup>th</sup>	✓ Does DMSO have any
Tag PCR buffer							2% contaminant?
% DMSO		1.6				1.6	✓ C <sub>f</sub> = 2% DMSO
23 min	1.91						✓ (-0.64 pmol 23 min / 10 <sup>3</sup> ) (= 6.4 nM primer)
1/2 5 min	-	-	2.4				✓ (note Vent buffer has 2 mM MgSO <sub>4</sub> )
5 mM KCl				4	8		
0.8 M NaCl	2						
U	66	64.4	69				✓
			63.7	59.7	55.7	66.5	

heat to 70°C, remove 10 µl at 2, 5, 15 min only, total 40

pol / circles

0.1 unit Tag = 0.005 pmol (per 10 µl Rxn)

0.064 pmol 23 min / 10<sup>3</sup> (= 0.464 nM total / 10 µl)

4 pmol circle  
5 pmol pol

0.012 3 ends / pol molecules

Expected units

0.1 U Tag gives 1 nM at 30'

have 0.464 nM at 23 min / 10<sup>3</sup> reaction volume

need ~14 min to replicate all DNA at least 1 unit / 30' (but not sure M13 gives same units)  
1 min would be ~500 at extension at unit value rates

compared to PCR

Tag / time

- This would be 0.5 units / 50 µl PCR
- 6.4 nM primer (20 10<sup>3</sup> times than 10 nM primer)

To Page No. \_\_\_\_\_

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Seena K. Polamp

Date

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Date

2-15-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

132

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100 bp ladder cut 10070-015

10  $\mu$ l H<sub>2</sub>O (vortex)

1  $\mu$ l 10 mCi/ml <sup>32</sup>P dCTP

15" 37°C  $\rightarrow$  10 $\times$  0.2 m EDTA

get total  $> 10^7$  CPM

load 0.2  $\mu$ l

$\frac{(20 \times \text{total})}{(20 \times \text{volume})}$

$10^7$  CPM  $\Rightarrow$  5000 CPM/ $\mu$ l

after 10  $\mu$ l EDTA

put

20  $\mu$ l

10  $\mu$ l

30  $\mu$ l

(Rxn + EDTA)

counts seq stop

$\geq$

300,000

10,000

CPM/ $\mu$ l

CPM/ $\mu$ l

30

load 1  $\mu$ l

T Pag N

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*Deena Polak*

Date

3/16/95

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*[Signature]*

Date

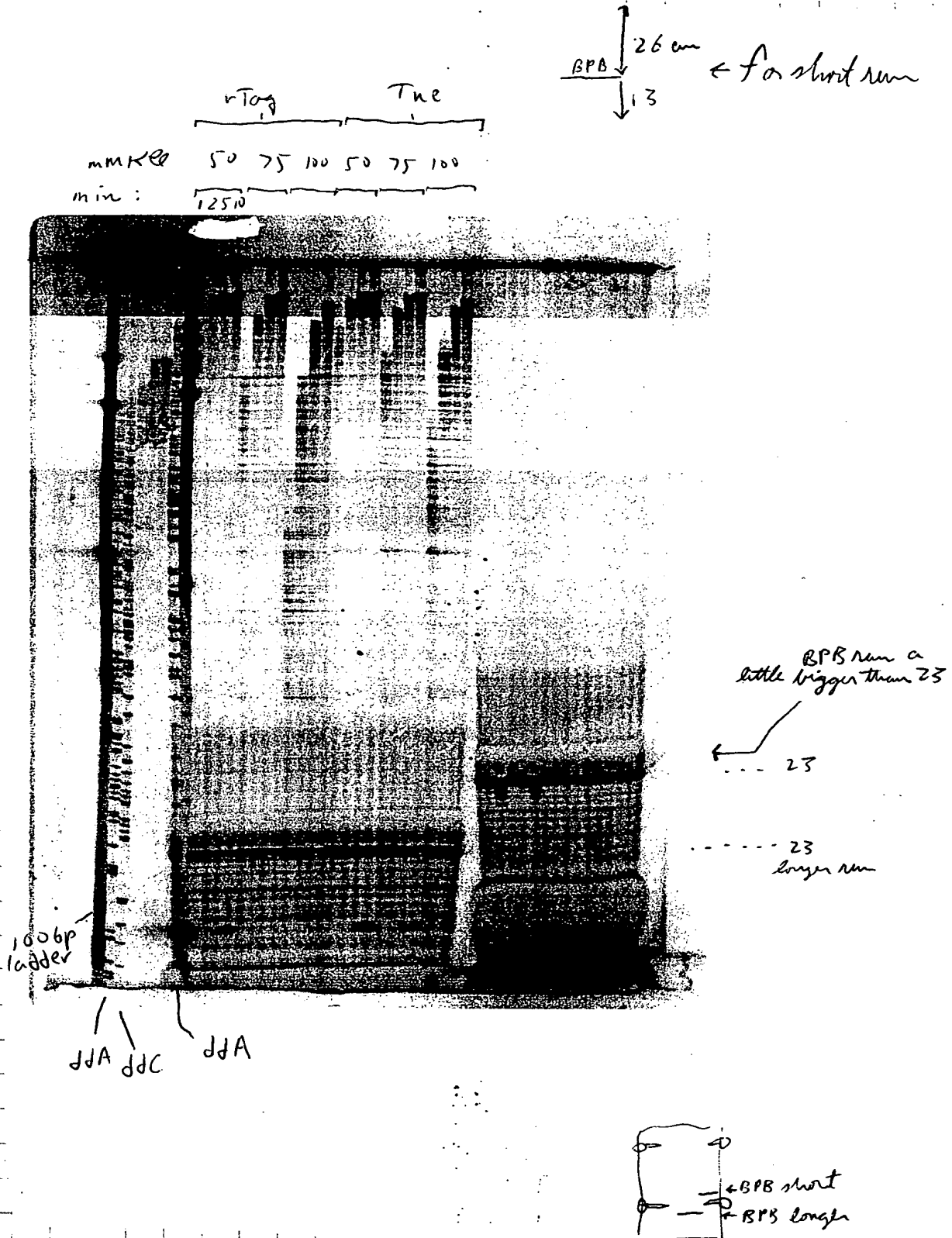
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Project No. \_\_\_\_\_  
 Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

134

From Page No. \_\_\_\_\_



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Date

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T Page N



136

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

primer 560826 (23mer with terminal A instead of G called "AC")

74.6 nmol total  
746  $\times$  H<sub>2</sub>O

CP = 100 pmol primer /  $\mu$ l (= 100  $\mu$ M primer)

Kinase

23mer "AC"	(100 $\mu$ M primer)	2 $\mu$ l	✓	✓	200 $\mu$ l
5x Kinase buffer	100 pmol 23mer / 1	8	✓	✓	23mer
32P $\gamma$ ATP 10mCi / ml	(3.3 $\mu$ M ATP)	20		✓	66 $\mu$ l
PNK 1u / $\mu$ l		2			
H <sub>2</sub> O		8	✓	✓	

30', 37°C  $\Rightarrow$  60°C, 5'

40  $\mu$ l CP = 5  $\mu$

use 2  $\mu$ l / 50  $\mu$ l PCR  
for 200 nM primer

note 1 unit T4 Kinase converts 1 nmol ATP / 30' at 37

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2-2095

ig N	1	2	3	4	5	6	7	8	9	10	11	12
x Tag PCR buff	5 $\mu$ l	→										✓
"AC" P136 (5 $\mu$ M)	2 $\mu$ l	→										✓
20 0.5 M	-	1	2	3	4	5	-	1	2	3	4	5
The 5 $\mu$ l →												
dilute to 2 $\mu$ l												
2, to 20 $\mu$ l												
water to 0.2 $\mu$ l →												
Ngell 2 50 mM												
H <sub>2</sub> O												
	10.5 $\mu$ l	→										✓
	59.53%	59.53%	59.53%	59.53%	59.53%	59.53%	59.53%	59.53%	59.53%	59.53%	59.53%	✓
	VF = 50 $\mu$ l											✓

(4 units total)

(0.4 units)

Cf = 1.5 mM

m M Kell Cf = 50 60 70 70 90 100 50 60 70 70 90 100

70°C, remove 10  $\mu$ l to 5  $\mu$ l stop at 20, 60, 120 min

Results on P13T

To Page No.

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see user Backup

3/16/95

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2-21-95

138

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

33 mismatches matched and mismatched

Fr m Page No. \_\_\_\_\_

11.64 nmol "33 correct"  
(primer # 5381 DGI (GOI))

582  $\mu$ l H<sub>2</sub>O

has correct G at 3' end at 1  
of R site in MCS G-33  
GAATTC  
20  $\mu$ M primer

13.42 nmol  
33 mismatches

671  $\mu$ l H<sub>2</sub>O

20  $\mu$ M primer

33 correct 1  $\mu$ M  $\times$  5.3  $\checkmark$   $\checkmark$  (5.3 pmol total)

33 mismatches 1  $\mu$ M 5.3  $\checkmark$   $\checkmark$

Y ADP (1.67  $\mu$ M ADP)  $\times$  4 4  $\checkmark$   $\checkmark$   $\checkmark$  (6.68 pmol)

5X Kinase buffer  $\times$  4 4  $\checkmark$   $\checkmark$   $\checkmark$

PNK 1  $\mu$ l 1 1  $\checkmark$   $\checkmark$   $\checkmark$

H<sub>2</sub>O  $\times$  5.7 5.7  $\checkmark$   $\checkmark$

20  $\mu$ l 20  $\mu$ l 37°C, 30'  $\Rightarrow$  5'

1 pmol

6.107 pmol circle  $\times$  mp19 0.2  $\mu$ g/l  $\times$  36.7  $\mu$ l 36.7  $\mu$ l

1M Tris pH 7.5  $\times$  0.6 0.6  $\checkmark$

H<sub>2</sub>O  $\times$  20.7 20.7  $\checkmark$

V<sub>2</sub> = 6.6 6.6  $\checkmark$

5', 95°C cool slow

use 2  $\times$  / 20  $\mu$ l reaction

= 0.6 pmol primer in 20  $\mu$ l

CP  
0.03 pmol  
primer  
0.06 pmol  
circles  
circle/  
= 2  
excess

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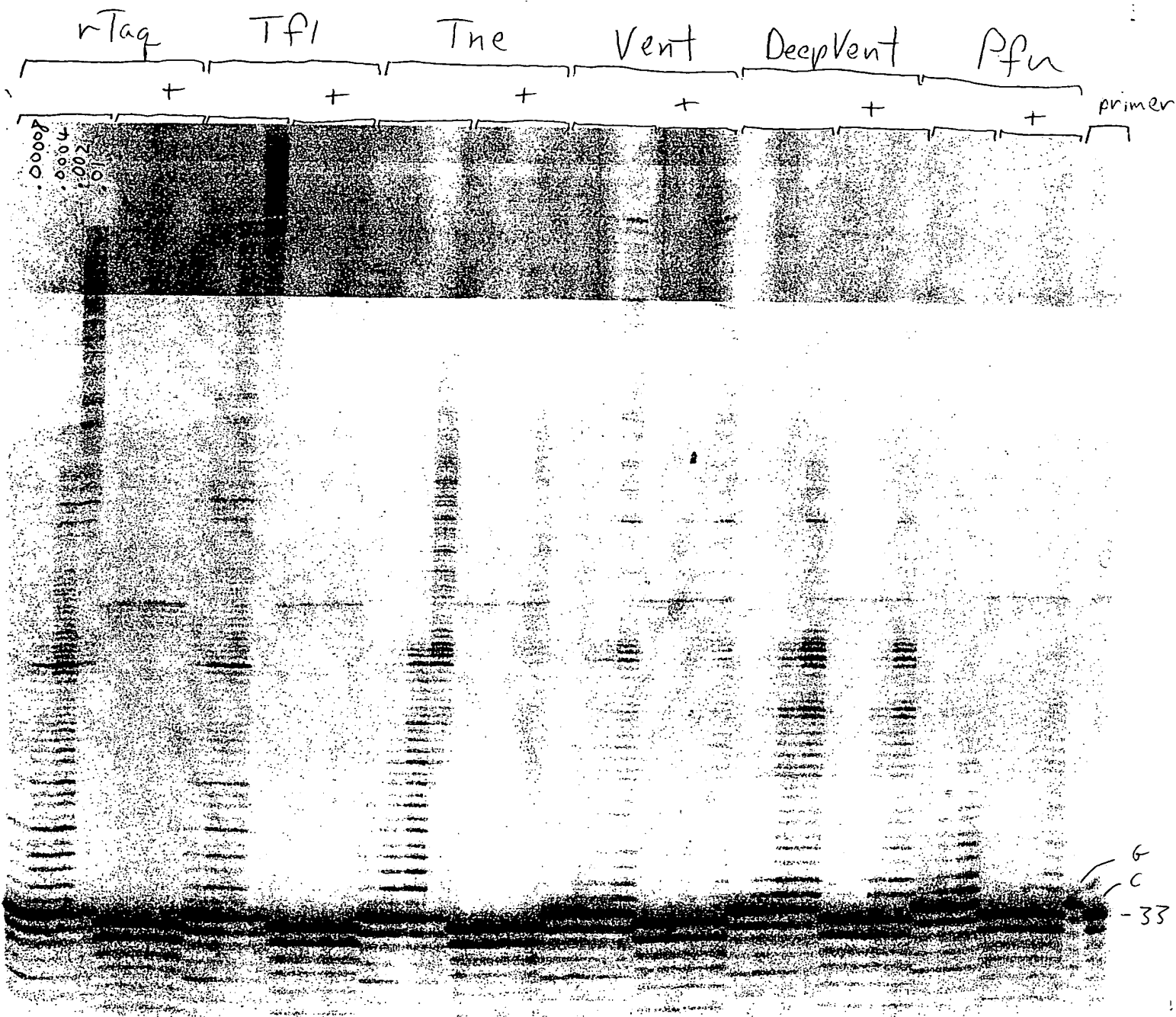
Date

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T Page 1

3/16/95

2-2395



6  
C  
-33

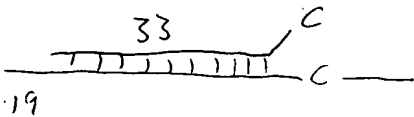
circles/pol molec

56

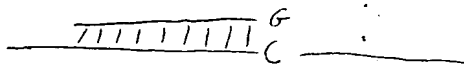
282

1410

7050

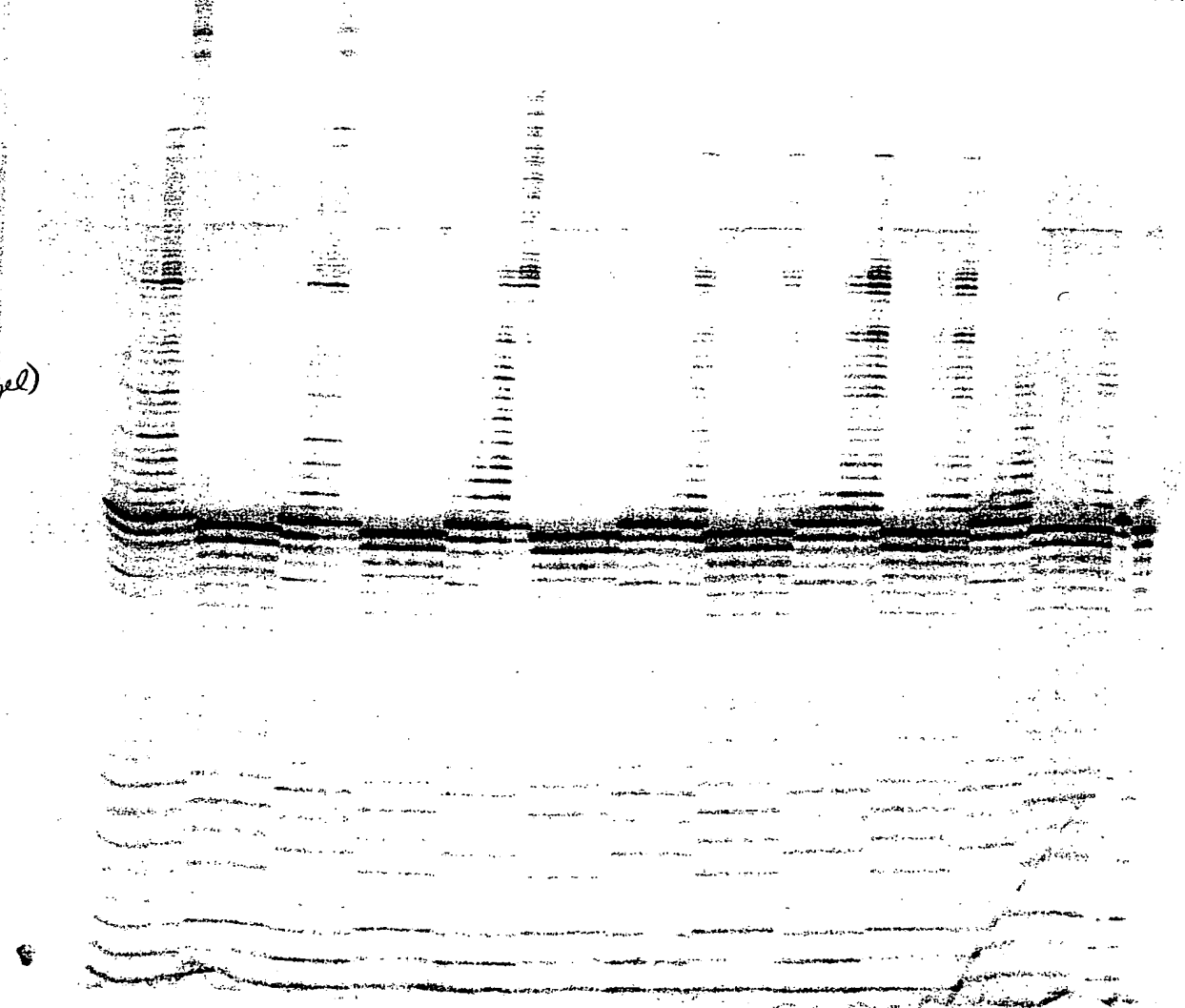
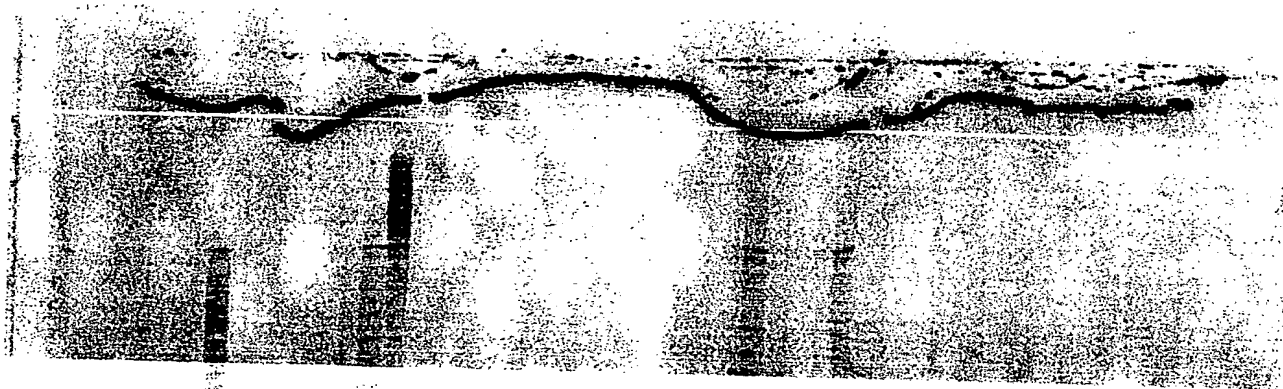


19





16% PAGE 33 watts, 4 1/2 hr  
 XC went 21.7 cm (Rf = 21.7/39 = 0.56)  
 33 mer migrated 25 cm  
 XC runs as a 40 mer



was in as 39 cm gel)  
 XC →

33 →

XC →

33 mer →

6 hr

↑  
 25  
 ↓  
 14  
 ↓

could run XC to ~ 30 cm

P  
 33 mer went  
 25 cm of 39 cm  
 gel long the

**R c r d d by**

2-7457

From Page No. \_\_\_\_\_

<sup>32</sup>P dNTPs - mp19 (P.138)<sup>32</sup>P mix - mp19 (P.138)

10mM dNTPs each

50mM MgCl<sub>2</sub>

10x PCR buffer

10x Vent buffer

10x Pfu buffer

H<sub>2</sub>OrTag .00008  $\mu$ l

-31-93 .0004

.002

.01

Tf1 .00008

-orienter .0004

-130008A .002

.01

The .00008

.0004

.002

.01

Vent .00008

.0004

.002

.01

DeepVent .00008

.0004

.002

.01

Pfu .00008

.0004

.002

.01

To Page \_\_\_\_\_

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Dat

3/16/95

Inv nt d by

Rec rd d by

Dat

20-24-55





Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Repair of 3' mismatch  
for TFI  $\pm$  Vent and rTag  $\pm$  DV, Pfu Tr

142

From Page No. \_\_\_\_\_

\*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

32 P33 mis-mplg  
(P138 2nd 0.01 pm primary/1)

2

10 mM JNTPs

5x Chug complete (4/14/95)  $\sqrt{4}$

0.4

5x chugach  
H<sub>2</sub>O

$\sqrt{13}$  12  $\rightarrow$  13 12  $\rightarrow$  13  $\rightarrow$  13.4 12.4  $\rightarrow$  13.4 12.4  $\rightarrow$  12.6  $\rightarrow$

TFI 0.1  $\mu$ /l

$\sqrt{1}$  1  $\rightarrow$

1  $\mu$ /l

$\sqrt{1}$  1  $\rightarrow$

Vent 0.02  $\mu$ /l

$\sqrt{1}$  1 1 1

0.01  $\mu$ /l

$\sqrt{1}$  1 1 1

0.05  $\mu$ /l

$\sqrt{1}$  1 1 1

rTag 0.5  $\mu$ /l  
5  $\mu$ /l

0.2  $\rightarrow$

0.2  $\rightarrow$

Deep Vent 0.02  $\mu$ /l  
0.05  
0.05

1 1 1  
1 1 1  
1 1 1

Pfu 0.002 0.005  
0.01 0.05  
0.05

Tne 0.022 0.01  
0.04 0.05  
0.05 0.1

(VF=20)

preheat all reactions to 70°C, start by addition of  
3' P33 mis-mplg, add 10  $\mu$ l cyd seq stop at 2 minutes

rTag, Tne TFI use Tag dil buffer  
Pfu, Vent, Deep Vent use NEB Vent dil buffer

T Pag N

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Dereasa Bolamp

Date

3/16/95

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R c rd d by

Dat

2-27-95

[illegible]

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Date \_\_\_\_\_

Inv nt d by

**Date**

**Recorded by**

2-27-51

146

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Δ KAc. Effect on pol and lxs, The v

From Page No. \_\_\_\_\_

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

5x Cheng (no KAC  
no DMSO  
no Glycerol)  
(at 5x = 100 mM Tricine pH 8.7,  
5 mM Mg(OAc)<sub>2</sub>)

✓ 4

KOAc 0.2 M

✓

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

33 correct. mpA (same as  
P13P. 0.06 pmol acid/λ)

✓ 2

32P 33 correct 5 μM primer  
(as was done for "AC" on 13.6)  
10 mM 4 dNTPs  
H<sub>2</sub>O

✓ 0.4

✓ 11.6 10.6 9.6 8.6 7.6 6.6 5.6 4.6 3.6 2.6 1.6 11.6 10.6 9.6 8.6 7.6 6.6 5.6 4.6 3.6

Tag 0.001 μ/λ

2

The 0.004 μ/λ

2

The 2.0 μ/λ

V<sub>f</sub> = 20λ

70°C, 5'

\* 33 correct has  
same 5' end as 23mer sequence primer

To Page N

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Deborah W. P. [Signature]

3/16/95

Recorded by

[Signature]

3-1-95

Page No. 27 28 29 30 31 32 33

→ ✓

2 3 4 5 6 7 8 9 10 ✓

ul → ✓

12	11	10	9	8	7	6	5	4
----	----	----	---	---	---	---	---	---

JX Chung on P 79:

20 mM Tris HCl pH 7.7

1.2 mM MgOAc

80% glycerol

20% DMSO

plus KOAC which is varied

from 0-100 mM in this experiment.

→

→

70°C, 60'

start 11.8

To Page No. \_\_\_\_\_

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Invented by

Kor

Date

3-1-95

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148

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

10x PCR same as P140  
TITLE is Tne inhibitory at T units ?

From Page No. \_\_\_\_\_

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
10x Tag PCR buffer	✓	2																			
<sup>32</sup> P 3300000 mp19 (P146) (see P138)		2																			
50 mM MgCl <sub>2</sub>	✓	0.6																			
10 mM DTPA	✓	0.4																			
H <sub>2</sub> O	✓	14											→ 13						→ 14		
r Tag																					
0.25 u/l	✓	1																			
0.5 u/l	✓	1																			
5 2	✓																				✓
5 3	✓				4																
5 4	✓				.6																
5 5	✓				.8																
Tne																					
0.25 u/l																					
0.5																					
5 2																					
5 3																					
5 4																					
5 5																					
Tag storage buffer	✓																				

preheat to 70°C, add 2 <sup>32</sup>P 3300000 mp19 for 30 sec  
kill with 10  $\mu$ l cyclo sig stop solution  
with 10 mM extra EDTA → 0.6 .4 .2 Cf = 20 mM EDTA in stop

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Date  
3/16/95

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R c rded by

Dat  
3-3-95

To Page No

Pr j ct No. \_\_\_\_\_

B k N . \_\_\_\_\_

149

ag N . \_\_\_\_\_

ed & Understood by me,

*mae a Polamp*

Date

*3/16/95*

Invent d by

Recorded by

Date

To Page No. \_\_\_\_\_

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

AatII #1

<sup>32</sup>P Kinase

150

From Page No. \_\_\_\_\_

see p 136 for ↑ [primer]

10  $\mu$ M AatII #1

5 ✓

5x Kinase

4 ✓

<sup>32</sup>P  $\gamma$  ATP 10 mM

10 ✓

(33 pmol ATP)

PNK 1  $\mu$ L

1

2  $\mu$ L

( $\Rightarrow$  now its 2.5  $\mu$ M primer)

37°C, 30'  $\Rightarrow$  80°C, 5'

↓

mix back into cold primer  
at 5 cold to 1 hot primer

<sup>32</sup>P AatII #1 2.5  $\mu$ M

13.3 20

(2.5  $\mu$ M)

cold AatII #1

16.6 25

(10  $\mu$ M)

30 45  $\mu$ L

(6.67  $\mu$ M)

(mg  $\text{MgCl}_2$  = 2.2)

use 1.5  $\mu$ L / 50  $\mu$ L PCR for 200 nM  
(adds 0.067 mM  $\text{MgCl}_2$  to PCR (f))

Ayoob R. used in 14 PCR's

remove 10  $\mu$ L from each PCR to 5  $\mu$ L stop (cp)  
and store at -20°C over weekend.

Result: Ayoob R.  
did PCR's with the

note smear (see EtBr stain  
(P151 photo) is not hot:  
30 primer ("AatII #1") is not  
needed for smear

To Page

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Date

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Date

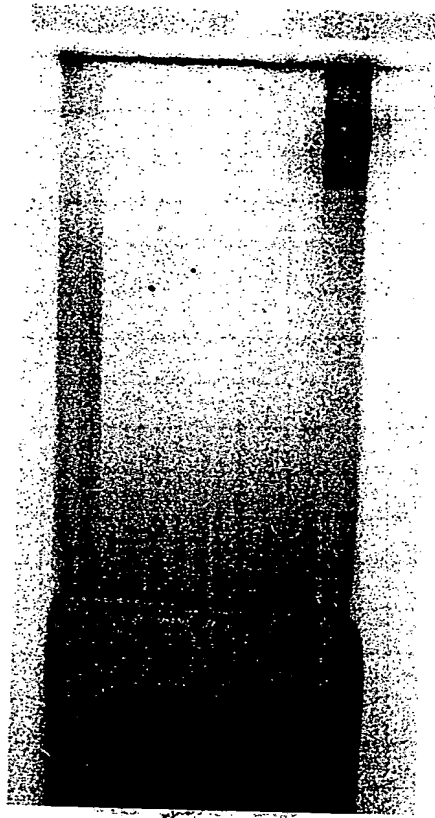
3-3-95

ag N \_\_\_\_\_

10% *agum* 180 V  
1 2 3 4 5 6 7 8 9 10 11 12  
1 1 2 3 3 4 4 5 6 6 7 7

0.5 x TBE

(lost 2nd, 5th)



To Page No. \_\_\_\_\_

Used & Understood by me,

*Michael Polansky*

Date

3/16/95

Invented by

Recorded by

Date

3-7-95



152

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Unit assay for stability of vTag in  
PCR mix. Repeat of assay on p 121

From Page No. \_\_\_\_\_

This assay is 33 days after the first assay of 2-3-95.

carry out all assays with exact same procedure  
of p 120 - 122, same Mag PL, TAPS, Kcl mix of p 121  
same stock of 'activated DNA', same 5' u/pl vTag stock  
on p 121 → (of 1-31-95)

(3' p d TP is a new stock of 10 mc/ml on 3-10-95)

T Pag N

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3/16/95

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Rec rd d by

Date

3-9-95  
3-8-95

relaxing to Tag  $\Rightarrow$   $\frac{u}{1}$

Pr j ct No. \_\_\_\_\_

B k No. \_\_\_\_\_

153

Exhibits

Activity	Activity	Activity	Activity	Activity	Activity
1	23165.00	} 24896	.031	.037	84
2	26508.00				
3	25014.00				
4	24738.00	} 24616	.031	.033	94
5	23608.00				
6	25502.00				
7	23947.00	} 23577	.030	.032	94
8	24449.00				
9	22336.00				
10	19450.00	} 19801	.025	.029	86
11	20001.00				
12	19953.00				
13	21103.00	} 22158	.028	.033	85
14	20211.00				
15	25159.00				
16	19309.00	} 18853	.024	.027	89
17	18318.00				
18	18933.00				
19	22404.00	} 23332	.029	.033	88
20	25483.00				
21	22108.00				
22	20542.00	} 23307	.029	.035	85
23	27602.00				
24	21776.00				
25	22624.00	} 22051	.028	.031	90
26	23813.00				
27	20017.00				
28	10829.00	} 11703	.015	.021	(70)
29	12483.00				
30	11798.00				
31	23967.00	} 24527	.031	.032	97
32	25056.00				
33	24557.00				
34	26587.00	} 25000	.032	.034	93
35	23432.00				
36	24980.00				
37	25401.00	} 24694	.031	.031	100
38	24104.00				
39	24576.00				
40	25123.00	} 25962	.033	.035	94
41	25545.00				
42	27217.00				
43	24143.00	} 23703	.030	.032	93
44	23491.00				
45	23474.00				
46	30440.00	} 31731	(-.04)		
47	31721.00				
48	30572.00				
49	32938.00	} 17377	.022		
50	32985.00				
51	17357.00				
52	17994.00	} 144943.00			
53	16781.00				
54	144943.00				
55	145358.00				

note #10 is not authentic. so add it to the at .01 to 20/MP4 each in Reaction mix

Issued & Understood by me, Deane Polay Date 8/16/95 Invented by R. Polay Date 3-9-95 Recorded by

Test of rule to use 1/600 Toz sol  
between 20-40 min after mixing

Standard Toz units array as per 120-120

10 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

be # 1,23 4 5 6 7 8 9  
tube # 1,23 4 5 6

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

# 10 11 12 13  
7 8 9 10 11 12 13

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 70 2hr 3hr

# 14 15 16 17, 18  
# 14 15 16 17, 18

make a standard 1/600 dil<sup>o</sup>

1797 µl Toz dil buffer  
3 µl 5 u/l Toz  
Vortex 5"

use immediately in triplicate for reactions 1, 2, 3 at  
0, 20 sec and 60 sec <sup>1.45 40 40</sup>  
also # ~~1797~~ 20 sit on ice + 2 3 hr before 10.1  
EDTA (is no time at 74°C to use if only activity at 0°C.

To Page No. \_\_\_\_\_

ss d & Understood by m ,  
Sandra Polansky

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3/16/95

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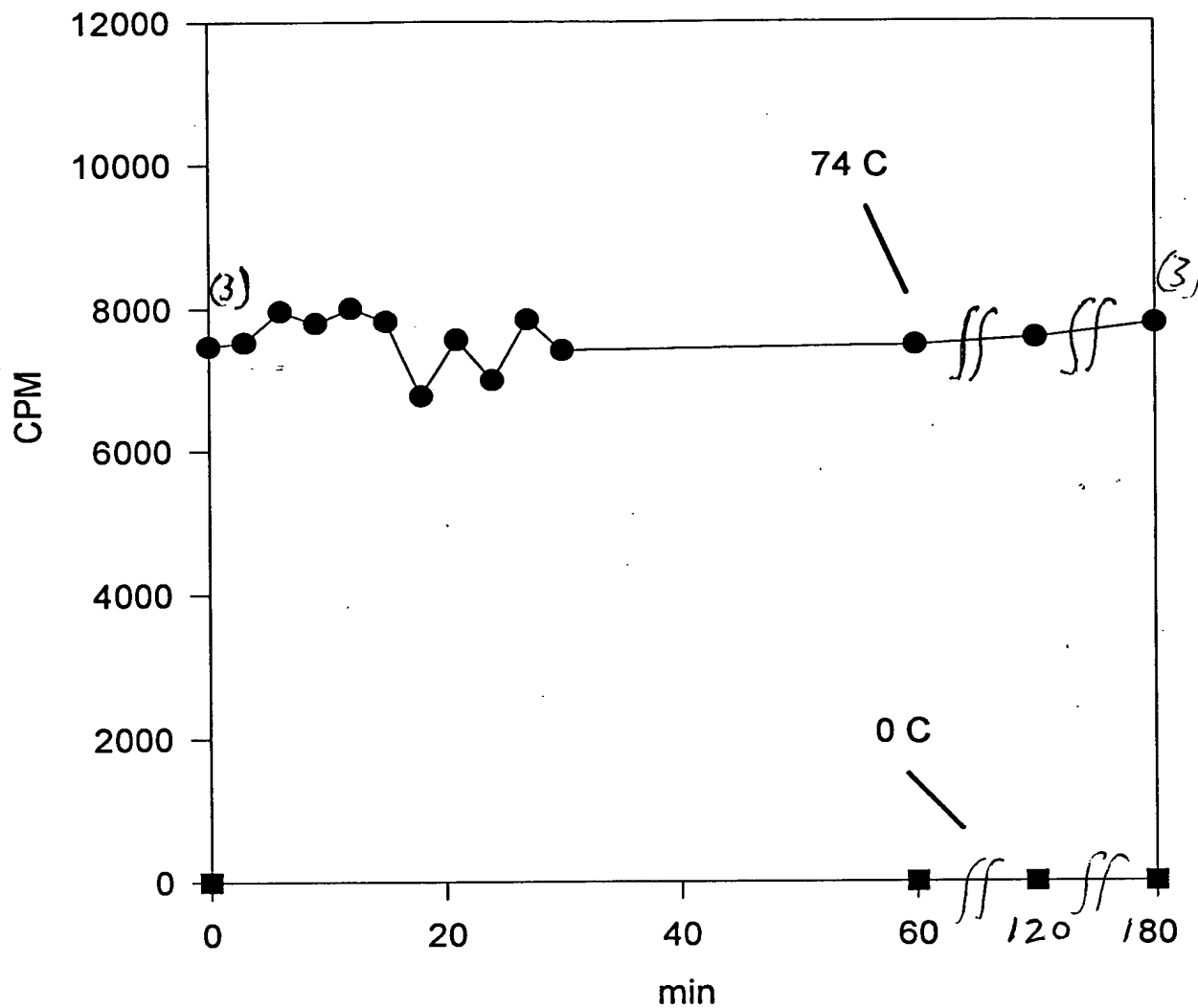
Date  
3-15-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

## Time allowed before assay of Taq dilution



SAM	CPM
1	738
2	845
3	705
4	770
5	809
6	802
7	820
8	796
9	692
10	764
11	733
12	801
13	760
14	970
15	765
16	784
17	750
18	828
19	827
20	27
21	66
22	40
23	59

Witnessed &amp; Understood by me,

*Deena Polansky*

Date

4/4/95

Invented by

Recorded by

Date

3-15-95

Tet stock / streak T+1 clones

Project No. \_\_\_\_\_  
Block No. \_\_\_\_\_

Exhibit L-54  
Appl. No. 09/558,421

157

Age N \_\_\_\_\_  
0.4g Tetracycline Sigma crystalline (not salt)  
40 ml ETH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol. ~15 ml agar  
to make 50 µg/ml Tet spread

15 µl 10 mg/ml Tet on each - let sink in ≥ 30 min  
25 50 µg/ml Tet in 35 ml agar plates

streak out cell (glycerol) stocks of AR

sup 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95  
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml will grow  
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at P:30

To Page No. \_\_\_\_\_

Read & Understood by me,

Carolina Polansky

Date

4/4/95

Invented by

Recorded by

Date

3-20-95  
3-21-95

Tet stock / streak T+1 clones

Project N \_\_\_\_\_

Block N \_\_\_\_\_

Exhibit L-55

Appl. No. 09/558,421

157

Page N \_\_\_\_\_

0.4g

Tetracycline

Sigma crystalline

(not a salt)

40 ml

ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar

To make 50 µg/ml Tet spread

75 µl 10 mg/ml Tet on surface - let sink in ≥ 30 min

25 µl 50 µg/ml Tet in 5 ml agar on plate

streak out cell (glycerol) stocks of A.R.

SUP 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml will grow + 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invent d by

Date

Deborah Polansky

4/4/95

Recorded by

3-20-95  
3-21-95

Tet stock / streaky T+1 clones

Project No. \_\_\_\_\_

Exhibit L-56

Appl. No. 09/558,421

Block No. \_\_\_\_\_

157

Page No. \_\_\_\_\_

0.4g

Tetracycline

Sigma crystalline

(not a salt)

40 ml

ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar

To make 50 µg/ml Tet spread

Typical 10 mg/ml Tet on water - let sink in  $\geq 30$  min

25 µg/ml Tet in 50 ml agar on plate

streak out cell (glycerol) stocks of A.R.

SUP 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95  
of each in which grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml which grow  
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No. \_\_\_\_\_

Assessed & Understood by me,

Deborah Polansky

Date

4/4/95

Invented by

Recorded by

Date

3-20-95  
3-21-95

From Page No. \_\_\_\_\_

3-22

30°C  
 Start 8:30  
 12:30 .274  
 2:00 .770

ASSD

↓ 42°C, 15 min

↓ 1 hr 37°C

3-23

extract and 55°C heat for FrI is  
 same as p 95 and p 115, 6

pol assay is same as PRT except add just  
 2 µl FrI' / 97 µl Rxn cocktail  
 and remove three points

assay 1 2 5 µl of FrI' in 50 µl  
 Top unit assay (using TFI buffer system)  
 for 5 min at 74°C

3-23-95

12-15-94

106	(100)	64
107 H	87	92
108 H	86	(100)
152	83	59
151	56	95
202	20	26
109	2	11

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Deanna Polak

Dat

4/4/95

Invnted by

Recorded by

Dat

3-23-95



Proj ct No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

158

From Page No. \_\_\_\_\_

3-22-

30°C  
Start 8:30  
12:30 .274  
2:00 .770

↓ 42°C , 15 min

↓ 1 hr 37°C

3-23-

extract and 55°C heat for FrI is  
same as p 95 and p 115, 6

pol assay is same as P95 except add just  
2 µl FrI' / 97 µl Rxn cocktail  
and remove three points  
array 1 2 5 µl of FrI' in 50 µl  
Top unit array (using TFI buffer system)  
for 5 min at 74°C

	<u>3-23-95</u> %	<u>12-15-94</u>
106	(100)	64
107 H	87	92
108 H	86	(100)
152	83	59
151	56	95
202	20	26
109	2	11

To Page No

Witnessed & Understood by me,

Deanna Polkamp

Dat

4/4/95

Inv nt d by

R cord d by

Dat

3-23-95

Repeat of 171-71: 141 clones  
except: induce 42° 15' → then 37°C

Proj ct N \_\_\_\_\_

Book N \_\_\_\_\_

159

Tag N		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
A (P95)		45	—																				
202 I'		1	2	5																			
106 I'					1	2	5																
107H I'								1	2	5													
108H I'											1	2	5										
109 I'														1	2	5							
151 I'																1	2	5					
152 I'																			1	2	5		
Fl. 0.1 u/l																							2

out buffer

43  
50 ml

43

43

43

43

43

43

3

5 min 74°C

SAM

CFM1

pmol u/ml

202	1	1	12102.00
	2	2	23777.00
	5	3	39574.00
106	1	4	63124.00
	2	5	79575.00
	5	6	108413.00
107H	1	7	52612.00
	2	8	76528.00
	5	9	107281.00
108H	1	10	51648.00
	2	11	71034.00
	5	12	100828.00
	1	13	1477.00
109	2	14	1552.00
	5	15	2682.00
151	1	16	33932.00
	2	17	57466.00
	5	18	90477.00
152	1	19	50494.00
	2	20	78908.00
	5	21	116569.00
0.1 u/l	22		3863.00
BF60	23		528.00
21	24		95707.00

37

.025

(expected .01 to 2.5x too high)

59.7

CFM/pmol

Result:  
get ~10X  
more activity  
if 37° instead  
of 42°C after  
the 42°C  
instructions

To Page No. \_\_\_\_\_

Used & Understood by me,

Date

Invented by

Date

*Deborah Pokany*

4/4/95

Recorded by

3-23-95

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE

5% PEI stock

160

From Page No. \_\_\_\_\_

Same as P155, 6 except ~~use~~ instead  
of using complete Tag ext buffer (P167, 3)  
just use 50 mM Tris HCl pH 7.5, 1 mM EDTA

A = 50 mM Tris pH 7.5 275 ml  
1 mM EDTA

(5526 UABR) PEI 50% 50 ml

ster  $\geq 30'$

adjust pH to 7.4 with HCl  
add A to  $V_f = 500$  ml

T Pag 1

Witnessed & Understood by me,

Date

Initialed by

Date

Deanna Bolamp

4/4/95

Received by

3-24-95

grow 2L TF1-106

Exhibit L-59  
 Project No. \_\_\_\_\_  
 B ok N . \_\_\_\_\_  
 Appl. No. 09/558,421

161

ag N .

make 2x LB (in 40g/L of LB with base  
 eg as per P 119, b for D. tok

make 20 ml O/N of TF1-106  
 in LB + 100 µg/ml Amp, 30 µg/ml Tet  
 (Manny L uses 15-20 µg/ml Tet)

10 mg/ml

Ampicillin (Sigma A-9518)

2 g

H<sub>2</sub>O

200 ml

filter sterilize

inoculate 10 ml O/N / 1L LB

start shaking at 30°C at P: 20 AM  
 start 8:20 AM ASD  
 12:30 PM 0.567

induce each at 42°C 15' - by rapidly  
 bringing up to 42°C in hot tap water bath  
 cool then 42°C in water shaker 15'

37°C 1 hr in air shaker  
 cool in ice water bath

end 1 hr 37°C at 2:05 and 2:35 respectively

OD<sub>550</sub> final = 0.812 ⇒ recovered 5.64g cells  
 (approx 5.1L 653 4 min)

To Page No. \_\_\_\_\_

Used & Understood by me,

Date

Inventor by

Date

Deena Polanco

4/4/95

Recorded by

3-26-95

3-27-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

162

5 buffers for 50g TFI prep

From Page No. \_\_\_\_\_

follow v Tag PRP 91342. PRP \* except for w  
2m KCl in  
buffer  
see.2L  
buffer B1L  
buffer C

1M K phos monobasic ✓ x 34.2

17.1 ml

1M K phos dibasic ✓ x 15.7

7.9 ml

glycerol ✓ x 160

80

KCl ✓ x 7.46g  
(50mm) d149.12  
(2m)

EDTA 0.5M x x 0.4ml

0.2 ml

BME 14.5M ✓ x 700  $\mu$ l350  $\mu$ lH<sub>2</sub>O 2L

1L

buffer C is 2mK  
here in order to de  
elution point - m  
in Tag PRP C,  
700 mm KCl

T Page N

Witnessed &amp; Underst od by m ,

Deanna Polanco

Date

4/4/95

Inv nt d by

R c rd d by

Date

3-27-95

AmSO<sub>4</sub> optimization for TFI  
(can see p 22, 7 for Tq)

Project No. \_\_\_\_\_  
B k No. \_\_\_\_\_

Exhibit L-61  
Appl. No. 09/558,421

163

ag N

3.64 g TFI cells (P161)

18 ml Tq ext buffer (P167,3)

sonicate

heat treat 75°C, 30 min

PEI

adjust to 200 mM NaCl  
Vol = 20 ml so add 1.33 ml NaCl 3M

add 5% PEI (P160) to C<sub>p</sub> = 0.4%  
stir 15 min (1.7 ml 5% PEI)

Centrifuge SS 34 15' 15 K

recover 17 ml supe = Fr I' / PEI

start 11:30 AM  
stir AmSO<sub>4</sub> in 15', spin  
SS 34 15 K, 15 min

	2 AmSO <sub>4</sub>	at salt
1 Fr I' / PEI	2.45 g	25
2	.433	30
3	.51	35
4	.51	40
5	.527	45
6	.527	50
7	.544	55
8	.561	60
9	.561	65
10	.578	70

To Page No. \_\_\_\_\_

Issued & Understood by me,

Date

Inv nted by

Dat

Deena Polanco

4/4/95

Record d by

3-29-95

164

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

*Pol assay of AmSO<sub>4</sub> supers*

From Page No. \_\_\_\_\_

*array 2 ml of 1/100 dil of each sups in 48  
ml R x n mix (P-120) for 15 min at 74°  
kill with 10% EDTA  
spot 40λ*

*Bradford*

*I / PEI*

*20*

*AS95*

*mg/ml*

*To Protein  
remaining*

<i>AmSO<sub>4</sub> 25%</i>		<i>.412</i>	<i>(0.39)</i>	<i>100</i>
<i>30</i>		<i>.450</i>	<i>0.40</i>	<i>102</i>
<i>35</i>		<i>.430</i>	<i>.38</i>	<i>97</i>
<i>40</i>		<i>.449</i>	<i>.40</i>	<i>102</i>
<i>45</i>		<i>.425</i>	<i>.38</i>	<i>97</i>
<i>50</i>		<i>.405</i>	<i>.36</i>	<i>92</i>
<i>55</i>		<i>.370</i>	<i>.33</i>	<i>85</i>
<i>60</i>		<i>.347</i>	<i>.31</i>	<i>79</i>
<i>65</i>		<i>.340</i>	<i>.30</i>	<i>77</i>
<i>70</i>		<i>.278</i>	<i>.25</i>	<i>64</i>
		<i>.242</i>	<i>.21</i>	<i>54</i>

*I / PEI / 70%*

*20/20*

*BSA 1 mg/ml*

*1  
2  
4  
6  
8  
10*

<i>.604</i>
<i>.105</i>
<i>.176</i>
<i>.263</i>
<i>.382</i>
<i>.474</i>
<i>.546</i>

*AmSO<sub>4</sub> 70%*

*CPM1*

*u/ml = 0.64*

<i>0</i>	<i>1</i>	<i>(11483.00)</i>	<i>(100)%</i>
<i>25</i>	<i>2</i>	<i>10706.00</i>	<i>93</i>
<i>30</i>	<i>3</i>	<i>11635.00</i>	<i>100</i>
<i>35</i>	<i>4</i>	<i>10329.00</i>	<i>90</i>
<i>40</i>	<i>5</i>	<i>7609.00</i>	<i>66</i>
<i>45</i>	<i>6</i>	<i>803.00</i>	<i>7</i>
<i>50</i>	<i>7</i>	<i>465.00</i>	<i>4</i>
<i>55</i>	<i>8</i>	<i>514.00</i>	<i>4</i>
<i>60</i>	<i>9</i>	<i>258.00</i>	<i>2</i>
<i>65</i>	<i>10</i>	<i>313.00</i>	<i>3</i>
<i>70</i>	<i>11</i>	<i>230.00</i>	<i>2</i>
<i>Blank</i>	<i>12</i>	<i>126.00</i>	<i>1</i>
<i>2λ</i>	<i>13</i>	<i>106668.00</i>	

*⇒ 66.7 cpm*

*3017 g cells  
(3.64g cells used)*

*conclude 45% AmSO<sub>4</sub>  
bring down > 90% min*

Witness d & Und rst od by m ,

Date

Inv nt d by

Dat

T Pag N .

*Deanna a Polansky*

*4/4/95*

R c rd d by

*3-30-95*

BSA

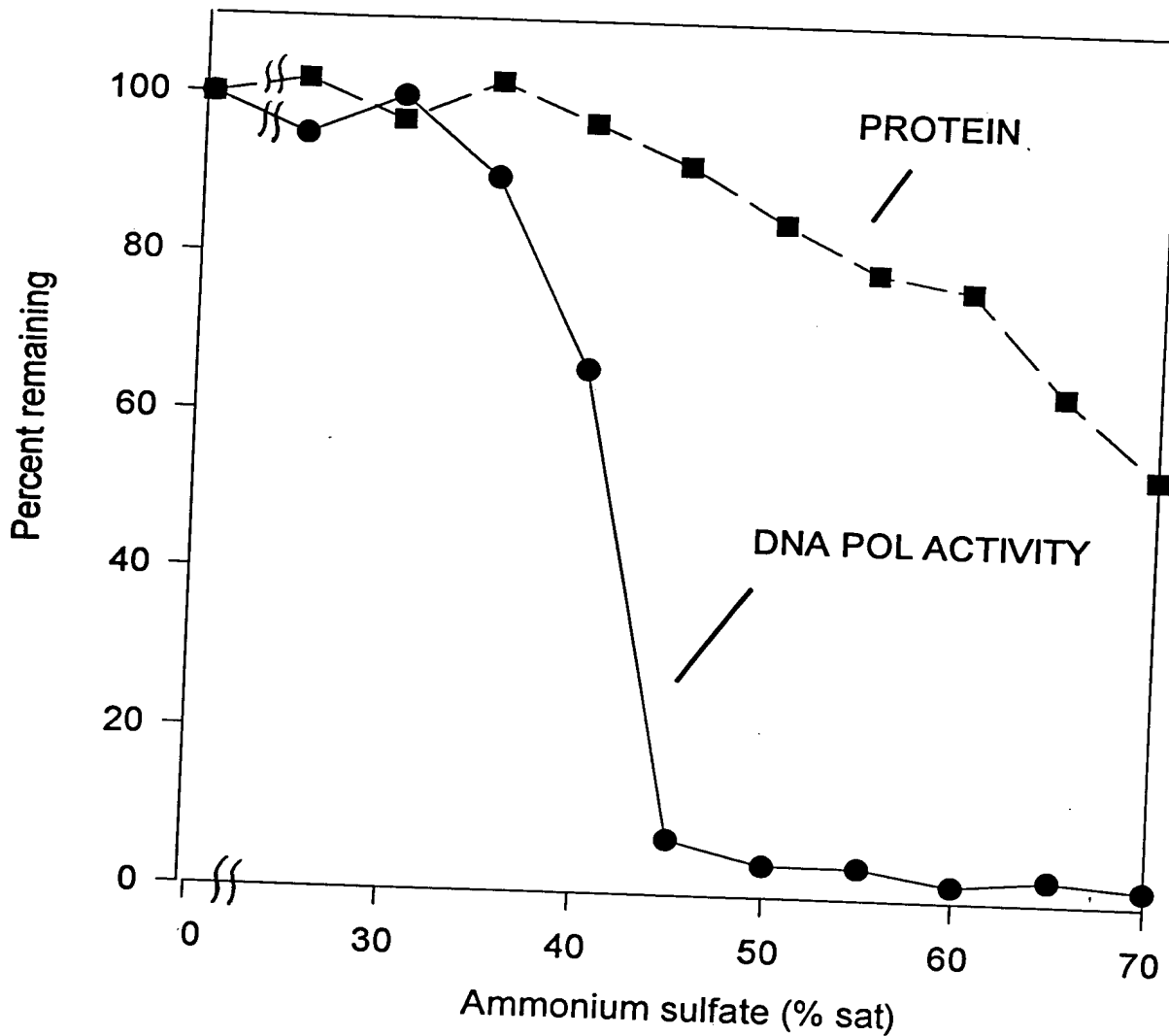
Pr ject No. \_\_\_\_\_

B ok No. \_\_\_\_\_

165

ag No. \_\_\_\_\_

## Precipitation of Tfi DNA polymerase



To Page N \_\_\_\_\_

Issued &amp; Understood by m ,

Dat

Inv nt d by

Dat

Recorded by



166

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE from a 180 ml sepharose 200

From Page No. \_\_\_\_\_

in a Mannin 2.6 XK

1. make slurry of cold sepharose 200 - want a  
1.5 x vol of paste vol

so 1.5 x 200 ml slurry = 300 ml

2. add 100 ml col buffer (buffer B p 162)  
so vol now = 2 x paste vol

and reservoir and gravity flow (got 50 ml/min with effluent tube 2  
below bottom of column and 100.  
in reservoir) -  $\approx 1/4$  vol vol/hr

bed volume ended up  $\approx 185$  ml (2.6 cm x 35 cm)

5 well 25 ml (bed vol) of Blue sepharose (Ph  
EL 65, in buffer B (p 162))

since dry swells 4 x 6.25 g

T Page N

Witnessed & Understood by m ,

Deena Polap

Dat

4/4/95

Invent d by

Record d by

Date

4-31-95

Stability unit assay for Targ  
 same as p121 and 122

Page N. \_\_\_\_\_

note stability study tube # 10 (unit assay # 27-30)  
 get 0.01% Taren 20/MP40 each added to reaction  
 by adding 0.5 ml of 1% stock

tube 51-56 =

Dextran	1.25 mg/ml	1 ml	Cf
	2.5	1	0.025
	5	1	0.05
	10	1	0.1
	10	2	0.2 mg/ml
	10	3	0.4
			0.8

51 19252.00  
 52 18303.00  
 53 18777.00  
 54 18582.00  
 55 17015.00  
 56 17487.00  
 57 267.00  
 58 104554.00

→ 65.3 cpm/pmol

To Page No. \_\_\_\_\_

Issued & Understood by m ,

Erica Polak

Date

4/13/95

Invented by

Recorded by

Date

4-85

From Page No. \_\_\_\_\_

SAM

CPM1

u/ml assuming  
rtay in .04u/ml in 1/1258610 to  
0.04u/ml P122  
8604

1	13329.00	.032	.037	8604
2	14243.00			
3	14542.00			
4	14132.00			
5	13839.00	.032	.033	97
6	13367.00			
7	14361.00			
8	14576.00	.033	.032	97
9	14684.00			
10	11765.00			
11	12054.00	.027	.029	93
12	11446.00			
13	13666.00			
14	13091.00	.030	.033	91
15	12913.00			
16	10381.00			
17	10049.00	.024	.027	89
18	10787.00			
19	16428.00			
20	14956.00	.034	.033	103
21	15556.00			
22	15357.00			
23	14468.00	.033	.035	94
24	13489.00			
25	14348.00			
26	12027.00	.030	.031	97
27	13354.00			
* 28	9416.00			
29	8913.00	.021	.022	100
30	9177.00			
31	13920.00	.032	.032	100
32	13672.00			
33	13373.00			
34	14628.00			
35	13728.00	.033	.034	97
36	15178.00			
37	14616.00			
38	14209.00	.034	.031	109
39	15366.00			
40	14402.00			
41	14584.00	.034	.035	97
42	15003.00			
43	12819.00			
44	13391.00	.030	.032	94
45	13180.00			
46	16169.00			
47	18733.00	17463 ave (.04u/ml) by definition		
48	18552.00			
49	16396.00			
50	12907.00			

\*fn #10, we .022 u/ml on P153 then  
dat added as 0.04u/ml point

To Page No.

Witness d &amp; Underst d by m ,

Date

Inv nt d by

Dat

D. C. R. Polans

4/13/95

R c rd d by

4-4-95

ag N	P12 in other point	1 month	2 months	4 months
1	.1% TN	84	86%	98
2	.2% BT	94	97	98
3	.2% TX	94	97	106
4	.01% TN	86	93	93
5	.02% BT	85	91	105
6	.02% TX	89	89	98
7	1% TN	88	103	104
8	2% BT	83	94	91
9	2% TX	90	97	99
10	No detergent	95	95	<del>95</del> -
11	1.1X	97	100	94
12	5X	93	97	100
13	2x R2GE 0.1%	100	109	(35)
14	2x Tff 1.01%	94	97	89
15	2x Vent	93	94	97

To Page No. \_\_\_\_\_

Used &amp; Understood by me,



Date

4/13/95

Invented by

Recorded by

Date

4-4-95

170

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Tf1 growth of 4-4-95

From Page No. \_\_\_\_\_

got ~ 0.6-0.8 g cells from 50ml samples taken at 0 1 2 3 4 hr post induction for 10L per of minimal media (001R) and the same for buffered rich (002R) plus 50ml at end (~4h post induction) for (002R) plus 114g bulk  
chopped off 0.55g of bulk for 4hr 002R sample

Resuspended cells in Tcy ext buffer (P167,3)  
 add 25 ml ext buffer  $\Rightarrow$  0.2 g cells/ml

sonicate 3 x 10 sec max setting microtip

microport 15 min, supe = Fr I

90°C 5 min

microport 15 min = supe = Fr I'

62.2  $\frac{\text{CPM}}{\text{pmol}}$   
see P167

pol assay is 2  $\mu\text{L}$  of 1/100 and 1/500 dil of Fr I'

hr after induction	pmol	$\mu\text{L}$	Graded Assay $\mu\text{L}$	mg/ml	$\mu\text{g}$
0	874.00	21	0.31	0.548	1.03
1	440.00				
2	6172.00				
3	1538.00	2.6	.677	1.28	2029
4	5174.00				
5	1058.00	1.8	.648	1.23	1467
6	6330.00				
7	1537.00	2.6	.670	1.27	2050
8	5734.00				
9	1206.00	2.1	.662	1.25	1675
10	1058.00	0.58			
11	324.00				
12	3961.00				
13	1227.00				
14	4250.00	2.1	.672	1.29	1626
15	1009.00				
16	4730.00	1.7	.700	1.33	1282
17	1046.00				
18	3435.00	1.8	.641	1.21	1485
19	763.00				
20		1.3	.734	1.27	1018

T Pag No.

Witnessed & Understood by me,

Deerana Polap

Date

4/13/95

Invented by

Record d by

Dat

4-5-95

114 g Thomas flr ~~cells~~  
mini g granlin

Proj ct No. \_\_\_\_\_ Exhibit L-66  
B k N . \_\_\_\_\_ Appl. No. 09/558,421

171

ig No. \_\_\_\_\_

cells 9504-02-767-03-002R  
(4 hr after induction)

Follow rTag PRP Document # 91342. PRP

114 gram cells

450 ml Tag extract buffer (buffer A)  
with fresh <sup>5mm</sup> BME + 50 µg/ml PMSE

~ 564 ml (30 ~ 0.2 g cells/ml)

one pass thru minigranlin 10,000 PSI

heat to 75°C ~~30~~ (~15')  
in 40°C water bath

15 min more at 75°C → cool in ice slurry

Adjust NaCl to 200 mM

have 550 ml Fr I' (ie after heating)

add 6.43 g NaCl

PEI adjusted to 0.4% by adding

47.8 ml 5% PEI pH 7.4 slowly, then  
ster 15 min more

To Page No. \_\_\_\_\_

s d & Understood by me,

Ernest Polansky

Date

4/13/95

Inv nted by

Recorded by

Date

4-7-95

Spin 30 min in GSA 13,000 RPM

J Ammonium sulfate

Recovered 506.6 ml of Fr I' / PEI

want 47.5%  $\text{Am}(\text{SO}_4)_3$  saturation

= 295.5 g / 1L

so add 149.7 g to 506.6 ml Fr I' / P

add slowly, stir 30 min more

centrifuge GS-3, 2500 rpm, 60 min

—  $\text{Am}(\text{SO}_4)_3$  pellet was coming off side of bottle  
 after 60 min spin  
 looks like pellet & solution

will try 2 hr at 15000 RPM in GSA

27000 g compared to ~12000 for GS-3

and smaller bottles (~150 ml / bottle in 4 bottles)

— result: pellets still floating

— collected ppts in filter and rinsed into 32 ml of clear filtrate

— spin 30 min in SS-34 18K

and spin 1 ml of 32 ml total in microfuge for unit assay.

ag N

Try diluting 1:1 the suspended  $\text{AmSO}_4$  ppt  
+ Try ext buffer lacking glycerol (ie 50 mM  
Tris HCl pH 7.5, 10 mM KCl) plus 47.5%  
saturated  $\text{AmSO}_4$ . is, the only effect is  
to reduce % of glycerol from 8% to 4%  
to see if ppt will pellet better

Result: ppt floats in 4% and also no glycerol!  
it does ~~not~~ sink in H<sub>2</sub>O

Result:  
see P 176 - cells induced only 1 hr don't  
have problem of  $\text{AmSO}_4$  pellets not sinking  
must be too many lipids in cells used here  
from 4 hr fermentation time point!

To Page No. \_\_\_\_\_

Read & Understood by m ,

*Carla Delamp*

Date

4/13/95

Invented by

Recorded by

Date

7-2-95



Project No. \_\_\_\_\_

Stability of Tay at room Temp

174

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

From Page No. — see P154, 3-13-91. Samples have been at room Temp 21  
 assay same as P121, 152.

0.5 M

TAPS 200 ml pH 9.3 (at room Temp)  
 (243.3 mW) (Sigma T<sup>cat</sup> - 5130)

24.33g + ~140 ml H<sub>2</sub>O  
 2M KOH to pH 9.3

H<sub>2</sub>O to 200 ml

tube # 1 - 30 is stability study 1E-15E in duplicate  
 note tubes 19, 20 (no detergent) gets 0.5ul of 1% Tween 20/R  
 24, 24 in the reactions.  
 (ie sample is stability study)

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by m ,

Dana A. Polak

Dat

4/13/95

Invented by

R c rded by

Dat

4-11-95

Results of #114  
 Crack TFI borne as P

Project N \_\_\_\_\_  
 B ok N \_\_\_\_\_

g N \_\_\_\_\_

	SAM	CPM1	ave	$\frac{a}{x}$	from TIME	of P121 values
	1	26896.00	26508	.04%	1.00	
	2	27150.00			1.00	
	3	26135.00			1.00	
	4	25462.00			1.00	
	5	26896.00			1.00	
1	6	22094.00	22048	.033	.037.00	89
	7	22002.00			1.00	
2	8	22874.00	22955	.035	.038.00	106
	9	23036.00			1.00	
3	10	21345.00	22335	.034	.032.00	106
	11	23325.00			1.00	
4	12	17420.00	17637	.027	.029.00	93
	13	17853.00			1.00	
5	14	19189.00	19840	.030	.031.00	91
	15	20491.00			1.00	
6	16	14064.00	14229	.021	.027.00	78
	17	14394.00			1.00	
7	18	19638.00	20655	.031	.033.00	94
	19	21673.00			1.00	
8	20	22693.00	20245	.031	.031.00	89
	21	17798.00			1.00	
9	22	17031.00	18271	.028	.031.00	90
	23	19511.00			1.00	
10	24	804.00			.022.00	0
	25	710.00			1.00	
11	26	17770.00	18729	.028	.032.00	88
	27	19687.00			1.00	
12	28	166725.00			.034.00	
	29	170523.00			1.00	
13	30	19772.00	19921	.030	.034.00	97
	31	20070.00			1.00	
14	32	21891.00	19376	.029	.031.00	83
	33	16862.00			1.00	
15	34	24156.00	22789	.034	.032.00	106
	35	21422.00			1.00	
BK00	36	1454.00			1.00	
21	37	134586.00			1.00	

To Pag No. \_\_\_\_\_

ed & Understood by m , <i>renera Poking</i>	Date <i>5/1/95</i>	Invented by <i>[Signature]</i>	Date <i>4.11.95</i>
Recorded by			

176

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Crack TFI same as P 171

From Page No. \_\_\_\_\_

These cells only grown for post induction

cells are 9504-10-767-03-003R

grown 4-11-95

10,000

resuspended 110 g cells in 440 ml (at room  
ext buffer (P167, 3) but no detergent  
PSI on minigoulin, 1 pass  
Bring to 75°C in 90°C water (~10 min)  
75°C for 15 min more.  
cool in ice slurry

Add NaCl to 200 mM Cf

Fr I vol = ~~510~~ 510 ml  
so add 5.96 g NaCl

add PEI (5% stock pH 7.4) to Cf = 0.

(used 0.4% last time (P 171) but want to get as  
as much DNA as possible

add 50.4 ml 5% PEI to 510 ml Fr I + N.

⇒ Cf = 0.45% add PEI dropwise and  
stir 15 min more

spin GSA 13,000 RPM 30'

recovered 49.5 ml sup ( = Fr I' / 1

Witness d & Underst od by me,

Domena Polans

Dat

5/1/95

Inv nt d by

R c rd d by

Dat

4-13-95

T Pag N

P116 continued  
Experiment done on P. 123

Pr j ct N .

Exhibit L-70

Bo k N .

Appl. No. 09/558,421

117

19 N

Still Needed 3

cut with Ord I to see if full length Sac Z is present  
(assuming either AfI<sup>III</sup> or Aa<sup>II</sup> recognition region  
had a point mutation/generator). Therefore the "410" and "465" bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 120

plus Aa<sup>II</sup>, AfI<sup>III</sup>

cut with 55<sup>+</sup> I to see if R1 site in MCS was  
a point mutation (or very small deletion  
(see on P107 at bottom) resulting in the "900mers"

miniprep # 3, 29

Recut with 17 $\mu$ l miniprep and load 30 $\mu$ l?

2.5 $\mu$ l reaction  
to try to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. \_\_\_\_\_

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Date

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Date

such a Polaris

2/16/95

R c rded by

1-31-95

From Page No. \_\_\_\_\_

resuspend entire AmS<sub>2</sub> pellet in buffer B (P.  
added 3 ml to ~ 1 ml pellet.  
triturate

spin SS34, 13 K RPM, 5 min

add ~ 200  $\mu$ l buffer B to pellet  
respin  $\rightarrow$  200  $\mu$ l buffer B more

need to microfuge 15 min to clarify

$V_f = 3.5$  ml (~~ca~~ 1.9% of 180 ml G100 col)

Load on 180 ml sephacryl 200

elute with  $\frac{1}{2}$  col vol/hr buffer B (ie 1.5 ml/min)

note mol started coming off  
column ~ 98 ml

98 ml / 180 ml col vol  $\approx$  54% col vol

Witnessed &amp; Understood by me,

Deanna Polans

Date

5/1/95

Invented by

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Date

4-18-95

To Page N

**PAGE 179 OF NOTEBOOK WAS BLANK**

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Standard  
TFI unit assay

From Page No. \_\_\_\_\_

mix used by epicenter same as Tq unit assay (P120)  
except only 160  $\mu$ g DNA/ml instead of 500  $\mu$ g  
in R<sub>2</sub>X

A  
0.5 M Tris pH 9.3 5.00  $\mu$ l  
1 M MgCl<sub>2</sub> 60  $\mu$ l  
3 M KCl 5.00  $\mu$ l  
V<sub>T</sub> = 2,060  $\mu$ l

"TFI R<sub>2</sub>X mix"

A 229  $\mu$ l ✓

10 mM dNTP 6.67  $\mu$ l ✓  
3.7 mg/ml DNA 144.2  $\mu$ l ✓  
10 mM  $\gamma$ -<sup>32</sup>P dCTP 6  $\mu$ l ✓  
H<sub>2</sub>O 2754  $\mu$ l ✓  
3.2 ml

use 48  $\mu$ l / 50  $\mu$ l reaction

T Page N

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Dat

5/1/95

Invented by

Dat

Record d by

Debra Polansky

4-1-95

2A 1/100  
Am 504 safe  
(2.4 m in  
Am 504 in  
reaction)

	SAM	CPM1	pmol	u/ml	total units
PET	1	4110.00	163	2.45	[1.21 x 10 <sup>6</sup> units in 495 ml]
Spent	2	6087.00		562.9	
4	3	308.00			fraction pool 7-12 = 18 ml total
5	4	356.00			
6	5	678.00			
7	6	3373.00			
8	7	8181.00			
9	8	11817.00			
10	9	9111.00			
11	10	8925.00			
12	11	5943.00			
13	12	2583.00			
14	13	1385.00			
15	14	773.00			
16	15	351.00			
17	16	299.00			
18	17	304.00			
19	18	245.00			
20	19	407.00			
21	20	2651.00	105	1.58	(expected only 1 u/ml in stock from epicentre)
22	21	358.00			
Sup	22	818.00			
Amix	23	60259.00			

37.7 cpm/pmol

for ~~fr 6~~<sup>7-12</sup> = average of  $\sim 8000$  cpm for 1  $\mu$ l  
 $\Rightarrow 47.7$  u/ $\mu$ l  $\Rightarrow$  759,000 total units / 1  $\mu$ l  
 or  $\sim 72\%$  recovery from Fr I' / PEI



Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Blue sepharose

182

From Page No. \_\_\_\_\_

load pooled sepharose 200 fractions #7-12 (18 ml V to  
on 20 ml Blue at 0.35 ml/min (~1 col vol)  
wash 5 col vol 0/N at 0.16 ml/min buffer  
gradient is 400 ml vol 50 mM - 1 M KCl  
(use buffer B-C - p162)  
at 3 col vol/min = 1 ml/min, 6 ml fraction

Buffer

in Tris pH 7.5  
0.5 M EDTA  
Glycerol  
3 M KCl

\* D

200 ml ✓  
1.6 ml ✓  
640 ml ✓  
2.8 ml ✓  
29.7g ✓

PC

(50 mM KCl)

E

25 ml 12.5 ✓  
0.2 ml 0.1 ✓  
PO ml 40 ml ✓  
0.35 0.2 ml 1.75 ✓  
14.9g 74.5g ✓

1E

500 ml

2 M KCl

(\*note buffer 0 is  
75 mM KCl in Tag Pop 71342)  
but only 50 mM here)

enter "2" to set to bank 2  
then 5

HOD 5 BANK 2

.00 CONC XB 0  
.00 CONC XB 0  
.00 ML/MIN 1  
.00 PPM DET

400 =  
400 =

still 1

a

To Page N

Used & Understood by me,

Date

5/1/95

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Date

4-15-95

Fig N. — pool Blue fractions 24-32 based on UV profile

$V_f = 54$  ml

Dialyze against 5L buffer D (PIB2) O/N  
recovered ~ 60 ml

Conductivity

10  $\mu$ l in 1 ml  $H_2O$

Buffer D  
in w/ effluent  
the equilibration O/N

10.1  $\mu$ S = 10.1 mS  
9.8  $\mu$ S = 9.8 mS

Dialyzate

9.9

9.9 mS

(can see P 41 where results are similar)  
for Toq

To Page No. \_\_\_\_\_

Used & Understood by me,

*serena Polansky*

Date

5/1/95

Invented by

Recorded by

Date

4-20-95

**PAGE 184 OF NOTEBOOK WAS BLANK**

# Heparin AF (20ml vol)

Proj ct No. \_\_\_\_\_

B ok No. \_\_\_\_\_

185

g N — Equilibrate O/N with buffer D, P182 (50 mM KCl) → see P183 for conductivity of col effluent  
 Load ~ 60 ml dialysate (P183) at 0.67 ml/min  
 = 2 vol vol/hr (as done on P11 for rTag)  
 (1 min/min)

wash ~ 1 vol vol 0.67 ml/min

for gradient want to make it fairly flat for first try  
 of TFI on Heparin.

Gradient:

50-700 mM KCl (= 0-35% pump B since  
 E is 2 M KCl)

20 vol vol = 400 ml, 4 ml/hr (so 100 portions total)  
 run at 2 vol vol/hr  
 so need 10 hours for whole gradient

rTag comes off Heparin ~ 400 mM KCl (see P 46)  
 so might see TFI ~ 6 hr from start ~ late afternoon  
 if TFI same as Tag

(loading done ~ 10:25 AM, wash 30 min (= 1 vol vol)  
 gradient start ~ 11 AM

100 5 BANK :

.00 CONC /B  
 .00 CONC /B  
 .00 ML/MIN  
 .00 PORT. SET  
 .00 PORT. SET  
 .00 VALUE. POS  
 .00 VALUE. POS  
 .00 CONC /B  
 .00 ML/MIN

To Pag No. \_\_\_\_\_

sed & Understood by m ,

erica Polay

Date

5/1/95

Inv nt d by

Rec rded by

Date

4-20-95

187

To Page No.\_\_\_\_\_

Date \_\_\_\_\_

4-21-JT

From Page No. \_\_\_\_\_

			<u>p.mol</u>	<u>w/pl</u>	<u>average w/pl</u>	<u>fraction</u>	<u>total units</u>
38	1	321.00	12		1.9		
39	2	526.00	20	3.0	3.0		
40	3	1566.00	60	9.03	9.9 ave		
	4	928.00		10.7		4ml	39600
	5	513.00					
	6	326.00					
	7	3904.00		22.52			
	8	1849.00		21.3	21.9 ave		
41	9	1346.00				4ml	87600
	10	792.00					
42	11	5730.00		33			
	12	3486.00		40	40.5	4ml	162000
	13	1668.00		38			
	14	1117.00		51			
43	15	5064.00		29			
	16	3156.00		36	41.3	2.68ml	110684
	17	1890.00		43			
	18	1239.00		17			
44	19	6029.00		34			
	20	3974.00		45.8	43.8	2.68	117384
	21	2233.00		51			
	22	969.00		44.7			
45	23	4489.00		25.5			
	24	2775.00		22	35.6	2.68	95408
	25	1960.00		45			
	26	858.00		39			
46	27	2156.00		12.4	12.3	2.68	32964
	28	1056.00		12.1			
	29	843.00					
	30	364.00					
47	31	847.00			4.9		13500
48	32	465.00			2.7		
Blue Box	33	4246.00					
Red Box	34	2441.00	93.9	14.0		54ml	756000
Diad	35	3795.00				60ml	
	36	2266.00		13.9			
	37	165.00					

after dialysis 751000  
30 10000 removed from dialysis

32.5 CFU/pmol

(occasionally sporadic)  
Opt. is extracted  
pmol fraction

Witnessed &amp; Und rsted by m ,

Dat

Invent d by

Dat

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Deena R. Shup

5/1/95

R c rd d by

4-21-95

ag N	units	% recovery
I'/PEI	1,210,000	100%
monium sulfate	1,280,000	100
phacryl 200	859,000	71
ue sepharose	756,000	62
alysis	751,000	62
arin AF	666,000	55

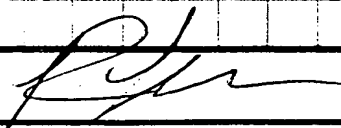
To Page No. \_\_\_\_\_

Used &amp; Understood by me,

Date

5/1/95

Invented by



Date

4-21-95

Recorded by



190

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

grow  $\lambda$  pL sp6 plasmid  
 in host lacking TFI sp6 plas  
 but containing Tet resistance  
 plasmid with  $\lambda$  promoter and  
 sp6 gene

From Page No.

Tet stock

LB 50 ml  
 Tet 30  $\mu$ g/ml  
 add stab of cells  $\rightarrow$  30°C shaking O/N  
 got no growth O/N ! 4-24  
 $\rightarrow$  as it just grew  
 slow - had cells 36 h

Repeat with M. Luzzi Tet and  
 at only 15  $\mu$ g/ml  
 after 36 h) grow O/N at 30°C  
 should dissolve "crystalline" TC in E  
 made fresh ~~TC~~ TC stock in H<sub>2</sub>O first

139 mg Segimen No T 3258 Tetraazoline  
 a little water  
 (it still doesn't go into solution)

100% ETOH (good stuff from Corcoran)  
 up to 27 ml  
 = 5 mg/ml stock in foil, -20°C

inoculate 1 ml of ON #5 into 50 ml  
 can circle grow + ~~15  $\mu$ g/ml~~ 30  $\mu$ g/ml of fresh TC stock  
 shake at 30°C

start 8:30 stop 3 PM got 0.22 mg cells  
 to add 0.88 ml Taget buffer (P167, 3) (for 0.2  $\mu$ g/ml)

Witnessed &amp; Understood by me,

Deena a Poling

Date

5/1/95

Invested by

Recorded by

Date

4-23-95

To Page N



# Regeneration of columns

Proj ct No. \_\_\_\_\_  
B ok No. \_\_\_\_\_

Exhibit L-76  
Appl. No. 09/558,421

191

Blue sepharose

2 col vol 6M Guanidini HCl  
5 col vol H<sub>2</sub>O (immediately)  
2 col vol 20% EtOH for storage

Heparin Af

2 col vol 4M urea  
2 col vol H<sub>2</sub>O  
2 col vol 20% EtOH

(Co. 3 = 0.5M NaOH recommended)

sepharose S200

1/2 - 1 col vol 0.4M NaOH  
contact with col =  $\geq 1 \text{ hr} \leq 2 \text{ hr}$ .

H<sub>2</sub>O 2 col vol  
20% EtOH for storage

run 0.4M NaOH at 2 ml/min  
for 45 min (= 1/2 col vol)

(start 10:20 AM) H<sub>2</sub>O for 3 hr at 2 ml/min  
= 2 col vol and NaOH only in  
contact with column for  
45 min x 90 min

20% EtOH 3 hr 0.2 ml/min O/N

To Page No. \_\_\_\_\_

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Date

5/1/95

Invented by

Recorded by

Date

4.28.95

192

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

SDS gel for TFI prep

From Page No. \_\_\_\_\_

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

DHIOBRK2

Fr I P190

3λ

Fr I' (75°C, 30) P190

30λ

TFI Fr I 2.45 u/l (P190)

3λ

Fr I' 2.45 u/l (P190)

30λ

AmSO<sub>4</sub> resuspended

0.5

3.62 u/l

5200 50 u/l

3.5

Blue pool Fr 24-32

12.5

14 u/l (P190)

Hepatic Fr #

39 3 u/l

5

40 9.9

5

41 21.9

5

42 40.5

5

43 41.3

5

44 43.7

5

45 35.6

5

46 12.3

5

47 4.9

5

TFI epimorph 1 u/l

30

cut TF31010A-502

2X sample buffer

30

H<sub>2</sub>O

27-27-3027 17.525

load 15 ul MW standards

CTI cut 10064-012

run at ~29 mA

started 9:15 AM

T Pag N ..

Witnessed & Understood by m ,

Deena Golap

Date

5/1/95

Invent d by

Record d by

Date

4-25-95

(180 ml)  
47 cm Heparin AF column

Project No. \_\_\_\_\_ Exhibit L-78  
Bo k N . \_\_\_\_\_ Appl. No. 09/558,421

1

ge No. \_\_\_\_\_

proved a 1.5 cm x 47 cm (180 ml) column  
to try to separate the 2 peaks on P186-187, 9  
flow rate is 0.204 ml/min by gravity.

gradient will be 50 mM - 400 mM  
and 10 col vol = ~~1600 ml~~ 1600 ml  
so gradient 1/2 as steep as P185, 9: 20 ml col

pool fr 40-43 (14.7 ml Vtotal)  
of Heparin (see P185-192, 9)

Dialyze ON against 1 L buffer D

(frms are ~ 300 mM KCl  
so expect ~ 4.2 mM + 50 mM in buffer)  
start gradient ~ 9:30 AM  
gradient is

1600 ml (20 col vol)  
50 mM - 400 mM KCl (was 50 mM - 700 mM on)

2 ml in 5 ml/min, so <sup>13.3</sup> 13.3 hr for gradient  
4.5 ~~hr~~ min/frn = 9 ml/frn (200 frms total)

note 1.5 ml/min gave only 0.2 mPa (column  
is definitely running with backpressure) but  
2 ml/min still only ~0.2 mPa so will  
use 2 ml/min  $\Rightarrow$  1.5 col vol/hr  
used 2 col vol/hr for 20 ml col P185, 9

To Page No. \_\_\_\_\_

sed & Understood by me,

maera P ooy

Date

5/1/95

Invented by

Recorded by

Date 4-26-95

4-27-95

From Page No. \_\_\_\_\_

expect protein to start coming off at  $\sim 65\%$  of the  
gradient. ie  $\sim 1040 \text{ ml} = 8.7 \text{ hrs}$   
or  $\sim 6:30 \text{ pm}$

since pool started coming off at  $13\% \text{ O} + 50 \text{ mM}$   
 $= 410 \text{ mM}$

### Comparison of 80 and 20 ml columns

col vol	20 ml	80 ml
col height	11 cm	47 cm
gradient vol	20 col vol	20 col vol
gradient stop	$\frac{35 \text{ mM KCl}}{\text{col vol}}$	$\frac{20 \text{ mM KCl}}{\text{col vol}}$
flow rate	2 col vol/hr	1.5 col vol/hr.

Therefore the new col is  $4\times$  longer, has  $0.75\times$   
flatter gradient and is  $0.75\times$  slower flow rate  
so hope to get better separation of 2 peaks see  
on P 186-187, 9

THOD 5 BANK 2  
 1.00 CONC XB C  
 1.00 CONC XB C  
 .00 ML/MIN 2.  
 .00 PORT.SET 3  
 .00 PORT.SET 6  
 .00 VALVE.POS 1  
 .00 VALVE.POS 2  
 3.0 CONC XB 20  
 1.0 ML/MIN 2.1

Witnessed &amp; Understood by me,

Date

Invented by

Date

Deeven Polarp

5/1/95

Record d by

4-27-95

**PAGE 3 OF NOTEBOOK WAS BLANK**

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

SOS gel on 80ml Heparin

From Page No. \_\_\_\_\_

I' DHIOBPK2  
P190

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19  
MW 200

MW  
150

I' TFI P1P1

200

AmSO4 resw 362 $\mu$ /A

1.5

5200 50 $\mu$ /A

145

Blue

62

With Heparin frms

105

150

106

150

107

150

108

150

109

150

110

150

111

150

112

150

113

150

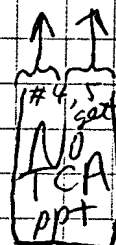
114

150

115

150

TFI 10+TF31010A-502



H<sub>2</sub>O

100 100 - - 238 150  
kf = 300

15% TCA

300 - - 300

P 72, 9 and P 50, 7)

30' ice

1 x sample buffer

35 50 - - 400 - - 35

T Page N

Witnessed & Understood by me,

Deborah Polys

Date

5/1/95

Inv nted by

R c rded by

Dat

4-28-95

**PAGE 5 OF NOTEBOOK WAS BLANK**

Storage buffer

Fr m Page No.

buffer F, G as per rtag 91342.PRP

make F, 4L first as follows.

make vol up to  $\frac{3200}{2700}$  ml ( $\therefore 80\%$  of V<sub>f</sub> of

remove	160 ml	and add
	20 ml	Tween 20 (Pierce)
	20	NP40 (Pierce)
ml	<u>200</u>	

= buffer G

Take the remaining buffer up to V<sub>f</sub> = 3200  
for 1X buffer Fpool frn 105-114 of Heperan (P. 1-4)  
= 90 ml vol (actually measured 87 ml)Dialyze against 2 buffer F, 5 hr  
2 ml L O/N

Recovered 33 ml after Dialysis.

combine with 33 ml buffer G = V<sub>f</sub> 66.

labeled:

TFI DNA pol in  
storage buffer 4-30-95

store at -20

4.33 ml  
SEP 8

Witnessed &amp; Understood by me,

Deena R. Rupp

Date

5/1/95

Inv nted by

R c rd d by

Date

4-29-95  
4-20-95

T Pag N



Storage buffer

From Page No. \_\_\_\_\_

buffer F, G as per rTag 91342.PRP

make F, 4L first as follows.

make vol up to  $\frac{3200}{2700}$  ml (i.e. 80% of Vf of

remove 160 ml and add

20 ml

Tween 20 (Pierce)

20

NP40 (Pierce)

ml 200

= buffer G

Take the remaining buffer up to Vf = 3800  
for 1X buffer Fpool frn 105-114 of Heperum (P. 1-4)  
= 90 ml vol (actually measured 87 ml)Dialyze against 2 buffer F, 5 hr  
2 ml L O/N

Recovered 33 ml after Dialysis -

combine with 33 ml buffer G = Vf 66

labeled :

TFI DNA pool in  
storage buffer 4-30-35

stock out - 20

4.33 ml  
all P8

Witnessed &amp; Understood by me,

Deena R. R. R.

Dat

5/1/95

Invent d by

Recorded by

Dat

4-29-95  
4-30-95

To Pag N

Unit assays for H1

ag N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
in storage buffer																											
4-30-95																											
sterile																											
250	2				2				2				2														
500		2				2				2				2													
1000			2				2				2				2												
2000				2				2				2				2											
lot 30809B																											
5 (see P181)																											
50																2											
100																	1										
200																		1									
400																			2								
F310/10A-502																											
4-25-95 P181, 9																											
0.5 gel																											
50																					2						
100																						2					
200																							1				
400																								2			
4-25-95																											
0.5 gel																											
50																									2		
100																										2	
200																											2
400																											2

Rxn mix 48  $\mu$ l  $\rightarrow$  48  $\mu$ l  $\rightarrow$

unit assays  $\rightarrow$  48  $\mu$ l  $\rightarrow$

74°C, 10',  $\rightarrow$  10  $\mu$ l 0.5M EDTA  $\rightarrow$  spot 40  $\mu$ l on GFC

Tubes # 13-16 are same serial dilution as tubes # 1-4  
made 3 independent serial dilutions in Tag dip buffer

To Page N \_\_\_\_\_

Used & Understood by me,

Debra A. Polansky

Date

5/1/95

Invented by

Recorded by

Date

5-2-95

From Page No. \_\_\_\_\_

	SAM	CPM1	<u>pmol-200 BKA</u>	<u>u/ml</u>		
dil 1	1	4167.00	102.8	3.85		
	2	2383.00		4.24	4.25 ave	
	3	1401.00		4.67		
	4	1060.00		(6.68)		
	5	4115.00		3.80		
dil 2	6	2316.00		4.1	4.38 ave	
	7	1394.00		4.6		
	8	846.00		5.0		
	9	4180.00		3.5		
	10	2416.00		4.3		
dil 3	11	1226.00		5.18	4.37 ave	
	12	888.00		5.3		
	13	4556.00		4.22		
Tag Rxn	14	2720.00		4.89	4.66 ave	
unt	15	1452.00		4.86		
(500 BKA)	16	1330.00		(8.28)		
	17	6287.00		1.17		
	18	4373.00		1.62		
	19	2428.00		1.73		
3000 B	20	1302.00		1.87		
	21	13554.00		2.59		
	22	8692.00		3.3		
1010 A	23	5151.00		3.8		
	24	3002.00		4.36		
	25	12756.00		2.44		
	26	8623.00		3.27		
1010 A	27	5397.00		4.06		
	28	2964.00		4.30		
BK 6-D	29	194.00				
= 200	30	96542.00				

5-2-ST label Hepair form of 4-30-ST  
at

4.33 units / A

T Pag N

Witnessed &amp; Understood by m ,

Deena S. Solari

Date

5/15/95

Inv nt d by

Rec rd d by

Date

5-2-ST

# Endonuclease Qc

Project No. \_\_\_\_\_

Exhibit L-84

Book No. \_\_\_\_\_

Appl. No. 09/558,421

9

follow + Tag Qc: 10342. QCP

22 Rxns	SS DNA Rxn mix	JS DNA Rxn mix	
PCR buffer M Tris HCl pH 8.4 20mM KCl	110 $\mu$ l	110 $\mu$ l	✓
2mM MgCl <sub>2</sub>	110	110	✓
174 (+) ssDNA 0.2 $\mu$ g/ $\mu$ l	110 $\mu$ l		✓
174 RF 0.33 $\mu$ g/ $\mu$ l		66.7	✓
il H <sub>2</sub> O	666 990	703.3 990	✓

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
4.30.50 3u/l	.48	.92	1.39	1.8	2.3												

spicula 10A-502 u/l (P8)	.57	1.14	1.7	2.3	2.9												
--------------------------------	-----	------	-----	-----	-----	--	--	--	--	--	--	--	--	--	--	--	--

crude xpt  
I 4-13-95  
il

1/10,000  
1/1,000  
1/100  
1/10

no del  
4.5 4 3.0 3.15 2.7 4.4 3.9 3.3 2.7 2.1 4 4 4 4 4 5 - ✓  
5

il buffer  
Vp = 50  $\mu$ l 72°C, 3 hr (start 12:10 PM)

1-17 is 45  $\mu$ l SS DNA Rxn mix and #18-34 is JS DNA Rxn mix 45  $\mu$ l Rxn mix

To Page No. \_\_\_\_\_

sed & Understood by m ,

Polcang

Date

5/15/95

Invented by

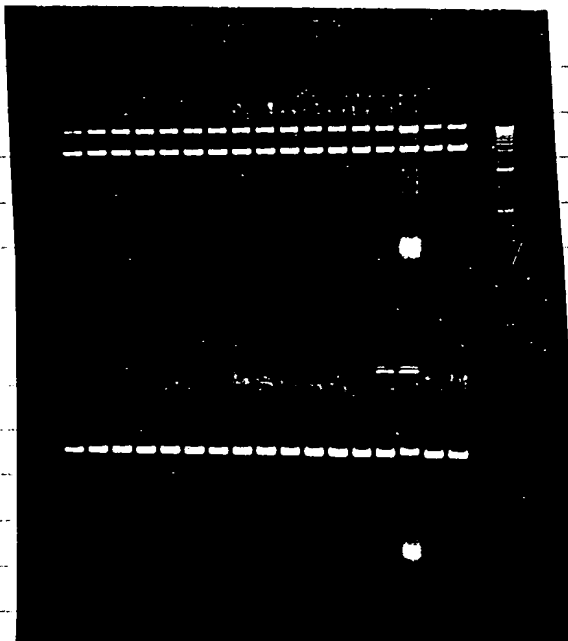
Recorded by

Dat

5-3-95

units: LTI TFI Epicenter / FrE  
 2 4 6 7 8 9 10 / 11 12 13 14 15 16 17 18 19 20  
 crude ext / Blank / dil buffer blank

(relaxed) RFII  
 SC DNA



} dsDNA  
 endr

} ssDNA  
 endr

note for RF II substrate with positive control (FrE)  
 there is a slight nick in relaxed circle (ie nick  
 and immediate conversion to small fragments.  
 no nicking seen for either ssDNA or dsDNA  
 substrates by any TFI or Epicenter.

To Page N

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Date

5/15/95

Inv. nt'd by

Recorded by

Date

5-3-95

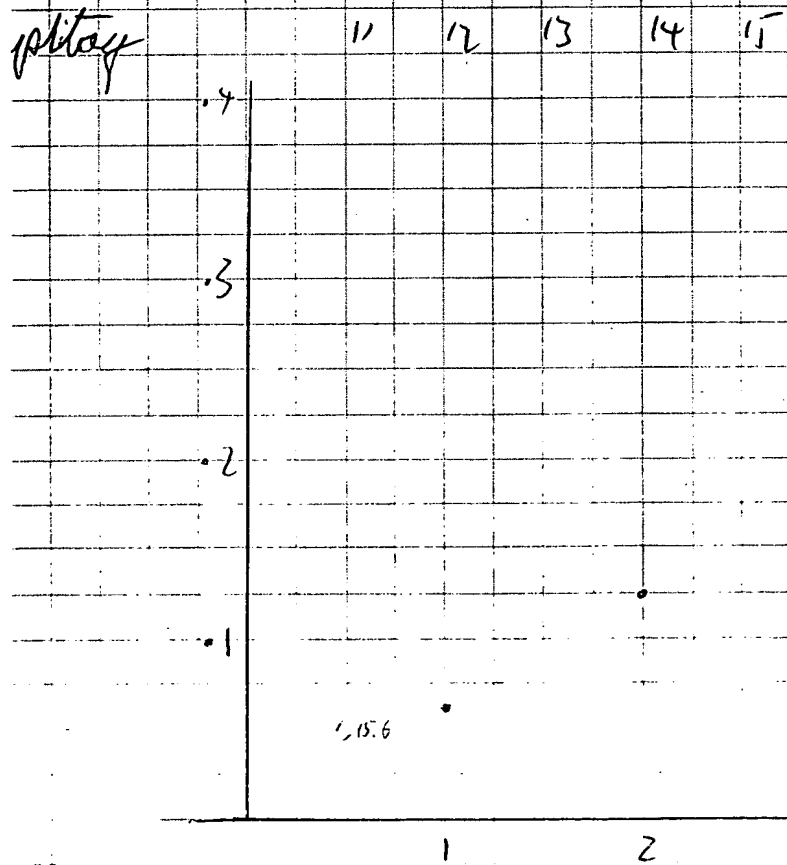
# Solutions of Tag for PCR functional assay

Project N. \_\_\_\_\_ Exhibit L-85  
Block No. \_\_\_\_\_ Appl. No. 09/558,421

11

Tube No.	#	1	2	3	4	5	1	2	3	4	5
CF u/ml		.0625	.125	.188	.25	.5					
lot #											
EM7414		5	5	5	5	5	3	3	3	3	4
g selection buffer		395	195	129.3	95	45	237	117	77	57	27
VF =		400	200	133.3	100	50	240	120	80	60	36

R401 # 6 7 8 9 10



To Page No. \_\_\_\_\_

Read & Understood by me,

*Polamp*

Date

5/15/95

Invented by

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Date

From Page No. \_\_\_\_\_

3' end reaction can be Cf = 200 nM

33 mer correct (P13P, 9)	20 pmol/l (20 µM)	7.5	15 µl	✓	✓	~ 300 pmol 33 mer
32P 2 ATP 10 mCi/µl		5	30 µl	✓		1.1 x 10 <sup>6</sup> cpm/µl
5-12-95		6	12 µl	✓		~ 900
5x Kinase buffer		1	3 µl	✓		
PNK 1 µl						
Hot						
		30	60 µl			
						37°C, 30'
						55°C, 5'

Plan for fidelity assay for pol ± 3' end

(33 correct P13P, 9)

(-) dCTP (+) dATGTP

CCAGTG A A T T G A G T G G T A  
 C T T A A G E T C G A G C C A T G G G C C C C

↑  
 same 5' end as  
 23 mer on mp19+

↑  
 only 3 into  
 for residue will  
 have to do

↑  
 most run through  
 stopped here for  
 quantitation  
 at 49-51

↑  
 n-1 is  
 3 into downstream  
 from primer  
 3' end

Witnessed & Understood by me,

*[Signature]*

Date

5/15/95

Invented by

*[Signature]*

Recorded by

Date

5-9-95

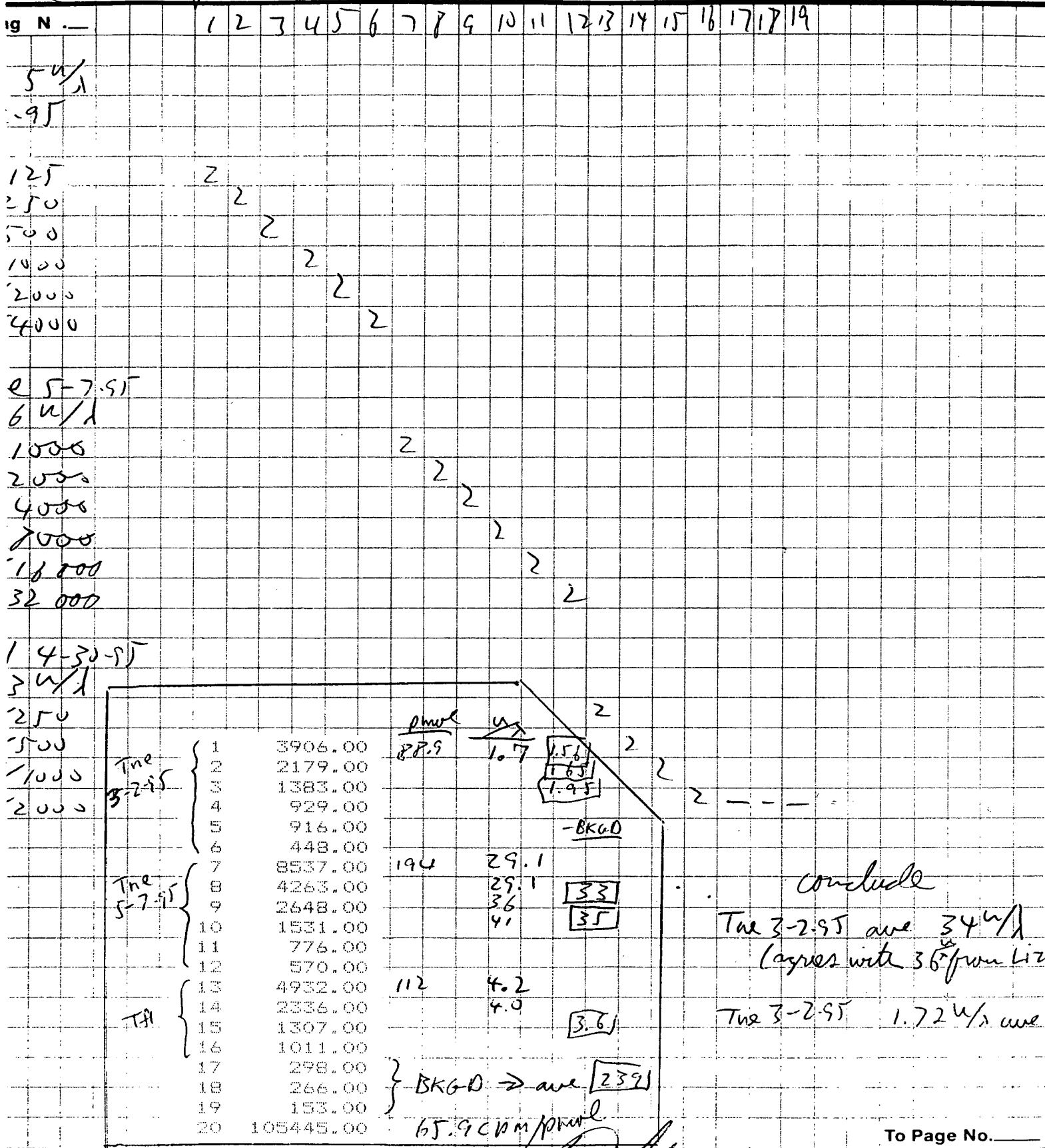
To Page 1

Unit array on 1he  
(in Tag unit array msp)

Project N. \_\_\_\_\_

Book No. \_\_\_\_\_

13



To Page No. \_\_\_\_\_

Designed & Understood by me,

*Bob Camp*

Date

5/15/95

Invented by

*[Signature]*

Date

5-5-95

Recorded by



From Page No. —

see P 136, 9 137, 9 for procedure: 200 nm primer (so its like a real PCR)

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪

cocktail

10x PCR buffer  
50 mM MgCl<sub>2</sub>5 —————→  
1.5 μl —————→60 μl ✓  
18 μl ✓ (1.5 mM)3'P 33mer correct  
P12 (5 μM)  
H<sub>2</sub>O

2 —————→

24 μl ✓  
102 μl — use 8.5 μl/

TFI FrI 4-1355

10

TFI FrI / PEI 4-1355  
(2.45 μl)

10

TFI in storage buffer  
4.33 μl (P.8)

10

for # (11)  
Chen  
✓ 10 μl of 5x buffer  
Nin (2-14.85 on  
has NTPA and 1TFI epimenter WATF31010A-502  
(3.5 μl P.8)

12.4

rTag 5 μl EKBT1

10

20 μl TFI / vent  
0.745 μl from 1The 3-245 1.72 μl  
(P.13) dilute to 0.5 μl

2/10

✓ 2 μl 3'P 33 mer  
✓ 18 μl H<sub>2</sub>O  
50 μl ✓

The 5-7-95 36 μl

2 10 —

(according to Liz F and see P13  
where I got 34 μl)

dilute to 0.5 μl

1% Tween 20 / NP40

H<sub>2</sub>O150  
150  
31 31 31 29.1 31.5 39.5 31.5 31.5 31.5 31.5

Tag dil buffer

10

74°C remove 10 μl to 5 μl crystal seq stop  
at 0.33, 1, 2 hr  
run on 8% PAGEsee analysis of TFI / vent exp rate and TFI loss of full length  
33mer on P 46.

Witnessed &amp; Understood by me,

Date

Initiated by

Date

Recorded by

To Page N

**PAGE 15 OF NOTEBOOK WAS BLANK**

From Page No. \_\_\_\_\_

10 PCR buffer

 $\frac{\text{mix A}}{110 \mu\text{l}}$ 

✓

Cp = 1X

acc

to

QC

50 mM MgCl<sub>2</sub>110  $\mu\text{l}$ 

Cp = 5 mM

1035

3 (Js) substrate

44  $\mu\text{l}$ 

(1 pmol/reaction)

0.5 pmol /  $\lambda$   
EXT EFE 73autoclaved, filtered H<sub>2</sub>O726  $\mu\text{l}$  ✓Vf = 990  $\mu\text{l}$ 

winds 0 2 4 6 8 10

tube # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

4-30-95

TF1 (P. 8)  
4.33  $\mu\text{l}$  /  $\lambda$ 

- 0.46 0.92 1.39 1.85 2.3

TF1 epicenter

EXT TF3 IN DA 502

3.5  $\mu\text{l}$  /  $\lambda$  (see P. 8)

- 0.57 1.14 1.71 2.28 2.86

TF1 Fr I

4-13-95

dil none 1/100

1/10

1/100

2

2

2

H<sub>2</sub>O 3 4.54 3.63 2.75 4.43 93.3 2.7 2.1

✓

Tag storage buffer 2

Mix A 45  $\mu\text{l}$ 

Tag dil buffer

Vf = 50  $\mu\text{l}$ 

5 3 →

✓

✓

✓

74°C, 60'

37°C, 60'

put tubes on ice

Witnessed &amp; Understood by me,

J. Polanco

Date

5/15/95

Invented by

Record d by

Dat

5-12-95

To Page N

# PET

Project N \_\_\_\_\_

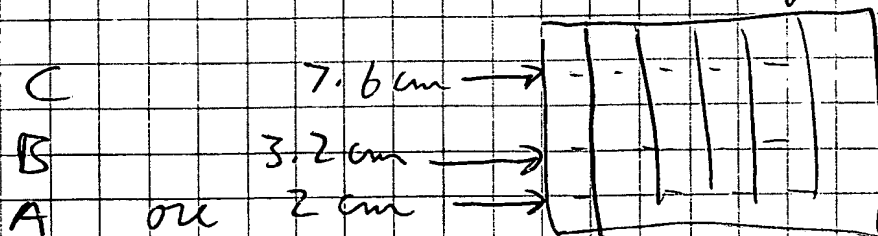
B k No. \_\_\_\_\_

17

g N \_\_\_\_\_ cut plate in half → get 20 x 10 cm plates

use 0.9 cm wide lanes  
origin at 2 cm

save lanes  
every 0.9 cm



spot 5  $\mu$ l, dry, spot another 5  $\mu$ l, dry  
resolve in 2 N HCl

bring solvent front to top of plate

Dry by heat lamp, not more than 7 min

count bottom - 3.2 cm (= ori)

3.2 cm = 7.6

7.6 = top

for each

3 x 12 = 34 tubes

add 3.5 ml flour

count 34

To Page No. \_\_\_\_\_

Used & Understood by me,

Polaup

Date

5/15/95

Invented by

Recorded by

Date

5-12-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

CPM

No released

TFI	4.30-50	0	ori	1	4802.00
			main	2	673.00
				3	92.00
				4	4782.00
		2		5	709.00
				6	123.00
				7	4935.00
				8	702.00
		4		9	114.00
				10	4670.00
				11	743.00
				12	129.00
		6		13	4732.00
				14	669.00
				15	123.00
				16	4788.00
		8		17	767.00
				18	123.00
				19	4661.00
				20	613.00
		10		21	84.00
				22	4677.00
				23	636.00
				24	145.00
TFI	picenter	2		25	4185.00
				26	767.00
				27	140.00
				28	4586.00
		4		29	774.00
				30	112.00
				31	4136.00
				32	887.00
		6		33	111.00
				34	4202.00
				35	793.00
				36	97.00
		7		37	1404.00
				38	396.00
				39	1801.00
				40	70.00
TFI	FrI	0.02X		41	133.00
				42	2536.00
				43	27.00
				44	208.00
		0.2X		45	2466.00
		2X			

BKGD

31

22

37

31

31

53

48

20

19

5

1709

2461

2374

0

0

0

0

0

0

0

0

0

0

50%

93%

91%

Conclude no ds 3'apo activity is detected in either LTI or Epicenter primed TFI pol

To Pag No

Witnessed &amp; Understood by me,

Date

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Date

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5-12-85

Page N. \_\_\_\_\_

Epicenter TFI storage buffer

LTI rtag storage buffer

Tris HCl

50 mM pH 7.6  
(pH at Room Temp based on Take call to Epicenter Tech line)

20 mM pH 8

need to increase molarity from 20 mM to 50 mM and pH from 8 down to 7.6

CaCl<sub>2</sub>

100 mM

0

need to add 100 mM NaCl to LTI SB

Glycerol

50 %

"

LTI Buffer as epicenter

DTT

1 mM

"

EDTA

0.1 mM

"

use 20/MP40

0.5 % each

"

Experiment

1 M Tris HCl

pH (room Temp)

10 ml buffer G

0

7.02 (expected 8.0)

5  $\lambda$

7.99

20  $\lambda$

7.92

20  $\lambda$

7.81

20  $\lambda$

7.77

20  $\lambda$

7.72

20  $\lambda$

7.67

20  $\lambda$

7.67

add 125  $\mu$ l of 1 M Tris HCl to 10 ml TFI 4-30-95

$C_f = 20 \text{ mM Tris (in SB)}$   
+ 12.5 mM Tris HCl added  
32.5 mM

will follow this exact procedure for 10 ml of TFI 4-30-95  
(pH 4.33  $\mu$ /l  $\Rightarrow$  new  $C_f = \frac{10.0}{4.33} = 2.31$

add 182  $\mu$ l 1 M Tris pH 7.5  $\Rightarrow C_f = 50 \text{ mM}$  pH = 7.60 pH

$V_f = 182 \mu\text{l} + 125 \mu\text{l} = 307 \mu\text{l}$   
plus 307  $\mu$ l glycerol  
 $V_{\text{total}} = 10.614 \text{ ml}$   
plus 62 mg  
mix end over end 30 to 60 min

NaCl 4 mM

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Boicamp

Date

5/15/95

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Recorded by

Date

5-12-95

$C_f = 100 \text{ mM}$  To Page No. \_\_\_\_\_

Stability study of  $\Delta$ in (no labeled)  
TFI / Vent mix (5-16-95)

From Page No. —

Follow p. 84, 9

[A]

5 x Cheng (no dATP)  
H<sub>2</sub>O  
activated DNA 3.7 mg/ml  
dATG-C-TP 10 mM ea  
32P dATP 10 mCi/ml  
5-19-95 w/ date 3000 Ci/mmol

200  $\mu$ l

637.9

135.1

5  $\mu$ l2  $\mu$ l

✓

✓

✓

✓

Cheng at IX

Tissue pH 9.0

100 AC.

DM50

mg AC

pH 2.0

2.0

85 m

2.02

1.05

0.5 mg/l

Cf 50  $\mu$ m $V_p = 780 \mu$ 

for 10 Rxns

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨

[A]

981 —————→

TFI LTISB 1.891 —————→  
+ Vent (5-16-95)

TFI Epicenter SB 2 —————→  
+ Vent (5-16-95)

TFI Epicenter Enzyme + Vent 1.89  $\mu$ l —————→  
(5-16-95)

 $V_p = 100 \mu$ 

68 °C

remove 10  $\mu$ l to 5  $\mu$ l 0.2M EDT  
(spot 10  $\mu$ l on 6-FC) and remove 5  $\mu$ l to 5  $\mu$ l Kill solution  
with cold dATP (P24, 9) and spot 2  $\mu$ l on PEI (P24,  
at 0, 10, 20, 40 min

Repeated on P. 40

Conclusion: There is no real good way to do this experiment because Vent is present at very low level compared to incorporation is saturated at levels where turnover is barely detected by PEI method. Will have to settle dDNA 3' end QC assay for units see p 26

To Page 1

Witnessed &amp; Understood by m,

Date

Investigated by

Date

D. [Signature]

6/9/95

R. [Signature]

5-11-95

*Turnover**Turnover*

ice No.

1247.00  
3736.00  
4880.00  
6046.00  
2170.00  
3243.00  
3897.00  
5694.00  
2774.00

*Experiments 179*

29 1925.00  
30 3149.00  
31 2983.00  
32 3730.00  
33 1091.00  
34 1772.00  
35 3000.00  
36 3722.00

3405.00  
4090.00  
5613.00  
3451.00  
3498.00  
5034.00  
3820.00  
2169.00  
2604.00  
2612.00  
4580.00  
2193.00  
2755.00  
5480.00  
4542.00  
2053.00  
3113.00  
2817.00  
3182.00

*incorp**pmol*

1 68601.00  
2 118176.00  
3 155582.00  
4 95344.00  
5 125044.00  
6 138326.00  
7 93269.00  
8 119376.00  
9 167655.00  
10 93777.00  
11 116666.00  
12 131003.00  
13 109619.00  
14 126936.00  
15 143456.00  
16 90599.00  
17 103792.00  
18 162204.00  
19 96493.00  
20 124924.00  
21 162506.00  
22 91166.00  
23 125191.00  
24 167630.00  
25 84292.00  
26 127063.00  
27 153977.00  
28 2135.00  
29 975.00  
30 110991.00  
31 110539.00

1487  
2562  
5372

RKOD  
RKOD

ave 110765 69.2 cm/p

To Page No. \_\_\_\_\_

sed &amp; Understo d by me,

*Edward Polap*

Date

*5/1/65*

Invented by

R corded by

Date

*5-16-65*



From Page No. \_\_\_\_\_

X buffer "S"

1M Tris HCl 7.5 pH  
 0.5M EDTA  
 glycerol  
 3 me  
 3M KCl

20 ml ✓  
 0.2 ml ✓  
 80 ml ✓  
 0.357 ✓  
 16.7 ml ✓  
 1 L

cf  
 20 mM  
 0.1 mM  
 8%  
 5 mM  
 50 mM

passed 6 ml S200

wash equilibrated at 1 col vol/hr  
 (= 0.1 ml/min) for 2 hr

load 120 ml of Tne 36 u/l 5-7-95  
 (= 2% vol vol) (4320 units total)  
 by gravity.

elute at 1 col vol/hr  
 into 95 fr

collect 50 ul frns (30 sec/fr)  
 span ~0.5(??)  
 A

\* with no detergent in buffer  
 maybe this is why activity died after  
 few weeks at 4°C (see P53)

Witnessed &amp; Understood by me,

Date

Invented by

Date

T Page 1

6/9/95

Record d by

5-17-95



g N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25																				
1/100	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27																				
Results on P 24																																													
9-95	unit array on fr 13-17 : pool 20 µl of each																																												
B-17	1	2	3	4	5	6	7	8	9	10																																			
100	2																																												
200	2																																												
400	2																																												
800	2																																												
1600	2																																												
3200	2																																												
5-7-95 3 u/l											134025	11	13	1	163.00																														
1000											23 Remin	12	14	1	216.00																														
2000											23 Remin	13	15	1	115777.77																														
4000											23 Remin	13	15	1	72%																														
8000											23 Remin	13	15	1																															
3	1	16254.83	338	5.1											<div style="border: 1px solid black; padding: 5px; display: inline-block;">10, 74°C</div> <p>will normalize 5200 Pool 13-17 against 36 u/l values for Tue 5-7-95 (see P 13) 00</p> <p>Pool 13-17 (7.7 u/l) / (56 u/l) = 3.9 u/l</p> <p>(3.9 u/l) (7 frms) (15 µl/frm) 1365 units recovered.</p>																														
4	1	12439.50	7.8																																										
5	1	5518.00	6.9																																										
6	1	2909.00	2.3																																										
7	1	1741.00	2.7																																										
8	1	866.00																																											
9	1	17122.00	355	53.2																																									
10	1	10433.3	45	6.5																																									
11	1	5683.00	70	70																																									
12	1	3137.00	47	77																																									

ave 7.7 u/l

70.7 ave

applied only 36 (see P 13)

To Page No.

To Page No. \_\_\_\_\_

sed &amp; Und rst od by me,

Polamp

Date

6/9/95

Invented by

Recorded by

Date

5-18-95  
5-19-95



3' exp (QC) array  
for TFI / vent

From Page No. \_\_\_\_\_

(see P20 where turnover on PET didn't work)  
follow array on P 16-17

actual units of Vent  
added based on 0.094 vent / 0.945  
TA/v

Mix A P. 16

5 1 2 3 4 5 6  
4545

TFI/vent LTESB  
(5-16-95)  
Tag 511 buffer  
2

~ 0.2

.02

.002

.0002

.00002

.000002

~~TFI/vent Epinephrine SB  
(5-16-95)~~

~~1/10  
1/100  
1/1000  
1/10000  
1/100000~~

~~TFI/vent with  
epinephrine TFI (5-16-95)~~

~~1/10  
1/100  
1/1000  
1/10000  
1/100000~~

H<sub>2</sub>O

3

BKWD 68°C, 60 min  
→ 88.00 21.31

BKWD start 2:35 pm

no dil	1	1684.00	4.87	1596
1/10	2	1323.00	5.49	1235
1/100	3	448.00	9.44	360
1/1000	4	143.00	16.71	55
1/10000	5	96.00	20.39	8
1/100000	6	101.00	19.92	13

Result: assay is not very h  
in any range  
with  
array at 1/10 and 1/100  
in triplicate dilutions

To Page 1

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Date

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Dat

Polans

6/9/95

Record d by

5-22-95

Project No. \_\_\_\_\_

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TITLE \_\_\_\_\_

*Results*  
*2nd of enzyme dilution*

28

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Front middle ori	413.00	4406	Sum		To last from one	Vend	282.00	Sum	3193 on		
	443.00		amt of triplicates				681.00				
	3550.00		mining 15K00				3216.00				
I SB	345.00	4050	314	244	15%	BKG0	252.00	Sum	an 7 32 41		
	557.00		520	199			623.00				
	3148.00		3275	-755			3170.00				
I SB	185.00	3874			15%	BKG0	85.00	4669			
	562.00						325.00				
	3127.00						3767.00				
I SB	308.00	4410			15%	BKG0	60.00	4669			
	524.00						317.00				
	3578.00						4292.00				
I SB	272.00	4216	302	229	15%	BKG0	469.00				
	603.00		552	231			271.00				
	3341.00		3434	-596			4391.00				
I SB	325.00				25%	BKG0					
	528.00										
	3383.00										
I SB	412.00				25%	BKG0					
	509.00										
	2885.00										
I SB	453.00	460	387		25%	BKG0					
	515.00		476	145							
	3004.00		2995	-1035							
I SB	515.00				25%	BKG0					
	434.00										
	3096.00										
I SB					25%	BKG0					
I SB					25%	BKG0					
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I SB											

Result  
5  $\lambda$  enzyme dil

sg	N	TIME	total sum	% lost from front	Result
1	front	324.00			<p>The sum of all counts when enzyme is present (ave 2879) is less than for the no enzyme blank (ave 3713) so 22% of counts unaccounted for!</p> <p>in each case ~ 1500 CPM is lost from origin (ie <math>^3H</math> removed by ep) but ~ 1/2 of that appears in front and middle. Also, why does any appear in middle it looks like <math>^3H</math> MP partly missing in middle since <math>^3H</math> runs in front, it may be quenched by broad contaminants that smear PEI at the front.</p>
2	middle	726.00	1.00		
3	ori	1780.00	1.00	2830	
4		348.00	1.00		
5	ave	960.00	1.00	3117	
6		1809.00	1.00		
7		458.00	1.00		
8		830.00	1.00		
9	1764	1703.00	1.00	2991	
10		483.00	1.00		
11		608.00	1.00		<p>43%</p>
12		1726.00	1.00	2817	
13		477.00	1.00		
14		515.00	1.00		
15		1698.00	1.00	2690	
16		288.00	1.00		
17		856.00	1.00		
18	1658	1670.00	1.00		
19		729.00	1.00		
20		627.00	1.00		
21		1452.00	1.00		<p>46%</p>
22		632.00	1.00		
23		511.00	1.00		
24		1199.00	1.00		
25		471.00	1.00		
26		686.00	1.00		
27	1354	1410.00	1.00		
28		374.00	1.00		
29		664.00	1.00		
30		1398.00	1.00		
31		200.00	1.00		<p>50%</p>
32		786.00	1.00		
33		1641.00	1.00		
34		128.00	1.00		
35		947.00	1.00		
36	1573	1682.00	1.00		
37		97.00	1.00		
38		477.00	1.00		
39		3007.00	1.00	3581	
40		66.00	1.00		
41		591.00	1.00		<p>57%</p>
42		3146.00	1.00	3803	
43		60.00	1.00		
44		480.00	1.00		
45	3122	3214.00	1.00	3754	
		1.00			

	2 $\lambda$ enzyme mix	5 $\lambda$ enzyme mix
LTISB	19%	43%
Epicate SB	15	46
Epicate TFI	26	57
Vert pol	21	50

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TITLE \_\_\_\_\_

30

Pool want assay for Nima TFI/Vent m  
same as P20 except used less units  
100 µl Tag unit assay P12, 9

From Page N \_\_\_\_\_

TFI Chemo unit assay m

H <sub>2</sub> O	1.056 ml	✓✓✓	35 Rxns
5X Chemo buffer	350 µl	✓✓✓	
activated OVA 3.7 mg/ml	236 µl	✓✓✓	(0.5 mg/ml)
JATCG-TP 10 mM ea	35 µl	✓✓✓	200 µM each
3-Phid CTP 10 mM ea/ml	3.5 µl	✓	
	1.68 ml		use 48 µl/Rxn

TFI Chemo unit  
assay m

48 µl →

med TFI 4 " #4 working stock

TFI 4.33 u/l (P.P)

1/250

2

2

1/500

2

2

1/1000

2

2

1/2000

2

2

TFI/Vent 5/16/5 LTISB

1/250

(P.P) TFI units

2

2

1/500

its ~4 u/l using

2

2

1/1000

my units (P.P) per TFI

2

2

1/2000

2

2

TFI/Vent 5/16/5 Epicenter SR

1/250

2

2

1/500

2

2

1/1000

2

2

1/2000

2

2

TFI/Vent 5/16/5 (Epicenter TFI)

1/250

1/500

1/1000

1/2000

74°C, 10'

dilute by adding 2.1 enzyme to 49.8 µl Tag dil buffer for

Witnessed &amp; Und rsted by m ,

Date

Invent d by

Dat

T Pag 1



6/9/95

R c rded by

1-2455



ig No. \_\_\_\_\_

		SAM	CPM1	(-B400)		
				<u>pmol</u>	<u>u/ml</u>	
				127	4.77	(was 4.33 on P8)
230 31 32 33	TFI	1	4165.00		5.14	
		2	2395.00		6.22	
		3	1575.00		6.9	
		4	1018.00		4.70	
		5	4108.00		6.7	
		6	3019.00		9.5	
		7	2232.00		7.7	
		8	1205.00		4.3	
	LTI SB	9	3788.00		5.0	
		10	2354.00		5.47	
		11	1425.00		7.62	
		12	1090.00		4.7	
		13	4135.00		5.0	
		14	2353.00		6.78	
		15	1688.00		7.07	
		16	1095.00		4.00	
	Epimeth SB	17	3543.00		5.06	
		18	2336.00		5.51	
		19	1431.00		7.13	
		20	1040.00		3.81	
		21	3388.00		4.64	
		22	2191.00		5.12	
		23	1354.00		5.31	
		24	857.00			
	Epimeth TFI	25	3746.00			
		26	2188.00			
		27	1548.00			
		28	866.00			
		29	4053.00			
		30	2474.00	71	5.3	
		31	1456.00			
		32	905.00			
	B/KGD Rx mix	33	322.00			
		34	72445.00	45.3	cpm/pmol	

will repeat this with 5 duplicates  
of the 1350 µl dil for each  
on P. 47

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I d &amp; Understood by me,

Solano

Date

6/9/95

Invent d by

Recorded by

Dat

5-24-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

units: 1.1x field test ("old")  
new, Basal M 2X mix

34

From Page No. \_\_\_\_\_

Tag 1-31-95

Su/pl

1/125

2

2

1/250

2

2

1/500

2

2

1.1x new (May 8, 1995)

no dil

2

2

1/2

2

2

1/4

2

2

1.1x old

no dil

2

2

1/2

2

2

1/4

2

2

BM 2X

1/2

2

2

1/4

2

2

1/8

2

2

-

Tag rxn

(P120, 9)

48 ml →

1-0 ml

74°C, 10'

see coded # P35

for Tag 1/25 at 2 ml undiluted of sample  
 new 6506 + 7080  
 Tag 10692 + 12403  $(.04 \mu\text{g Tag by def}) = .023$

6729 + 6660

10692 + 12403

023

Witnessed &amp; Understood by me,

Date

Invented by

Date

To Page

Polay

6/9/95

Rec rd d by

5-25-95

ag N \_\_\_\_\_

		pmol	u/μl		
1/125	1	10692.00	412	7.7	
1/25	2	5333.00		7.7	
1/125	3	3112.00		8.0	
	4	12403.00		8.9	
	5	6387.00		9.2	
	6	3853.00		10.0	
1/2	7	6505.00	250	.038 u/μl	1.77 u
1/2	8	4314.00		.050	2.50
1/4	9	2381.00		.047	2.39
	10	7000.00		.040	2.0
	11	4401.00		.051	2.5
	12	2364.00		.047	2.4
	13	6729.00		.039	1.9
	14	3962.00		.046	2.3
	15	2247.00		.041	2.2
	16	6660.00		.038	1.9
	17	3659.00		.037	1.9
1/2	19	3456.00		.036	.87
1/4	20	1705.00		.032	.77
1/8	21	1368.00		.047	.77
1/2	22	3028.00	1.7	.035	.84
1/4	23	2005.00		.039	.94
1/7	24	900.00		.026	
	25	333.00	BKGD		
	26	62215.00	37.9 cpm/pmol		

7.7 is ~ right since this  
"5 u/l" is normalized to  
amplitude that was ~8 u/l

Total units in  
in 50 μl  
at 1.1x

\* in 50 μl at 1x

average

\* 1.76

2.22

\* 1.75

1.88

0.86

~ red is average  
total units in 50 μl  
at 1x for the array of  
undiluted μl of 1.1x mix  
so use 1.76 units for "new"  
and 1.75 units for old "field test"

\* mix is 1.1x  
so at 1x mix is  
(u/μl in 1.1x)

as my first three points for these samples. (note expect ~2  
and dilutions above indicate 2.22u and 1.79u for old and new)  
see next array on P52 which is 1 month time point  
using 2 μl undiluted

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s d & Understood by me,

Bolano

Date

6/9/95

Inv nted by

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Dat

5-25-95

36

Proj ct No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

unit array for stability  
array of 1.1 x 10<sup>6</sup> (p121, 9) (at  
base of 1.1 x 10<sup>6</sup> of 1.1 x 10<sup>6</sup> 2300 time from

From Page No. \_\_\_\_\_

1	8878.00	.036	98	2300 time from
2	9039.00			
3	9623.00			
4	8220.00	.032	98	
5	8228.00			
6	8109.00			
7	8855.00	.034	106	
8	8307.00			
9	8584.00			
10	6857.00	.027	93	
11	7096.00			
12	6660.00			
13	9295.00	.035	105	
14	8535.00			
15	8519.00			
16	6780.00	.026	98	
17	5930.00			
18	5879.00			
19	8250.00			
20	8545.00	.034	104	
21	9288.00			
22	8605.00			
23	7590.00	.032	91	
24	7975.00			
25	7909.00			
26	7993.00	.031	99	
27	7301.00			
28	2863.00	.012 <sup>u</sup>		
29	3151.00			
30	3188.00			
31	7926.00			
32	7626.00			
33	7355.00			
34	8180.00			
35	8930.00	.034	100	
36	9000.00			
37	2662.00			
38	2885.00	.011	35	
39	2632.00			
40	8091.00			
41	7872.00	.031	89	
42	7722.00			
43	7664.00			
44	7828.00	.031	97	
45	8063.00			
46	10091.00			
47	9701.00			
48	10062.00			
49	10476.00			
50	10230.00			
51	101.00			
52	56411.00			
53	57488.00			

see you  
result.  
on p1

repeat in  
section  
p153

will  
(repeat)

10112 ave  $\Rightarrow$  42 bpm  $\Rightarrow$  7.99 units/sin  
(expect 27 units 54/1 EKBT1  
normalized to amplitude of 28

T Pag N.

With ssed &amp; Understo d by me,

Polay

Dat

6/9/95

Invented by

R c rd d by

Dat

5-26-95  
5-26-95

Stability of 1:1 X at room temp

ag N

0 time on P. 154, 9 3-13-55  
 1 month P. 174, 9 4-11-55

assay same as P. 121, 9 for 4°C stability study  
 used same assay mix as P. 5-25-52

amp slit #	Reaction tube #	ul enzyme P. 2x100	1% Tween 20 NP40	Tag unit assay mix (P. 121, 5)
	1-3	2		48 $\mu$ l
	4-6	1		
	7-9			
	10-12			
	13-15			
	16-18			
	19-21			
	22-24			
	25-27			
	28-30	↓	0.5 $\lambda$	
	31-34	3.64		
→ dil 1/2.5	34-36	2		
	37-39	1		
	40-42			
	43-45			
trubility	46-48 47		0.5 $\lambda$	
	48-50 49			
5 $\mu$ l 1-31-55	52-56	↓		
5 dil **	50-52			
	53 2 $\lambda$ of "old mix"			
	54 2 $\lambda$ of second mix			

74°C 10  
 kill with 10  $\mu$ l  
 0.5M EDTA  
 spot 40  $\mu$ l  
 on 6 FIC

10% TCA, 1% NaPP  
 ↓  
 3 x 5' in 5% TC  
 1 x 5' in 95% EtO

12  $\mu$ l Tag dil buffer + 8  $\mu$ l sample #12  
 \* 5  $\lambda$  tag + 620  $\lambda$  dil buff

Issued & Understood by me,  Polamp	Date 6/9/95	Invented by 	Date 5-30-95
		Recorded by	

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TITLE \_\_\_\_\_

From I

From 1	1	3864.00	.024	.037	2 months 65%	1 month 79
1	2	4350.00				
	3	3113.00				
	4	155.00				
2	5	130.00	0.00090	0.033	2.7%	106
	6	133.00				
	7	4121.00				
3	8	4864.00	0.029	0.032	91%	106
	9	4225.00				
	10	3184.00				
4	11	3267.00	0.021	0.029	72%	93
	12	3240.00				
	13	3952.00				
5	14	3728.00	0.025	0.033	76%	91
	15	3817.00				
	16	2585.00				
6	17	2492.00	0.017	0.027	63%	78
	18	2795.00				
	19	4009.00				
7	20	4530.00	0.028	0.033	85%	94
	21	4334.00				
	22	4199.00				
8	23	4281.00	0.028	0.031	90%	99
	24	4326.00				
	25	4248.00				
9	26	3739.00	0.026	0.031	84%	90
	27	3922.00				
	28	65.00				
10	29	86.00	0.00053	0.022	0.24%	0
	30	93.00				
	31	4340.00				
11	32	3827.00	0.026	0.032	81%	78
	33	4038.00				
	34	5155.00				
12	35	5666.00	0.035	0.034	103%	—
	36	5301.00				
	37	3927.00				
13	38	3981.00	0.026	0.031	84%	97
	39	4352.00				
	40	4214.00				
14	41	3962.00	0.027	0.035	77%	73
	42	4287.00				
	43	3992.00				
15	44	3958.00	0.026	0.032	81%	106
	45	4278.00				
	46	2014.00				
40°C	47	2016.00	0.013			
40°C	48	1504.00				
40°C	49	1509.00				
Total	50	5941.00	} (6.04 w/1) by definition 61.77 same as P36 when corrected for 30µl on GFC instead of 40µl and 4 day decay of <sup>230</sup> P			
	51	6122.00				
	52	6468.00				
	53	49708.00				
	54	40962.00				

T Pag N

Witnessed &amp; Understood by me,

Date

Inv nt d by

Date

R cord d by

Page No. \_\_\_\_\_

sp act

$$\frac{\left( \frac{46819 \text{ CPM}}{0.000 \text{ pmol dCTP}} \right) (4)}{29.3 \text{ CPM/pmol}} = 29.3 \text{ CPM/pmol(nt) DNA}$$

$$\left( \frac{6177 \text{ ave Tag CPM}}{29.3 \text{ CPM/pmol}} \right) \left( \frac{60 \lambda}{30 \lambda} \right) = 421 \text{ pmol DNA synthesis}$$

1 unit 10 nmol / 30' at 74°C

$$\left( \frac{.421 \text{ nmol}}{10 \text{ mol}} \right) \left( \frac{30'}{10'} \right) = 7.89 \text{ u/mol}$$

u of sample =  $\left( \frac{.04 \text{ u/mol in Rxn}}{7.89 \text{ u/mol for Tag}} \right) \frac{\text{samples CPM}}{\text{Tag CPM}}$

200 μM each dNTP

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6/9/95

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5-30-95

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Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Repeat of P. 20 : stability of  
TFI / Vent mixes

40

From Page No. \_\_\_\_\_

H<sub>2</sub>O  
5x Cheung buffer (no dNTPs)  
activated OMA 3.7 mg/ml  
ATG-C-TP 10 mM each

44.6.4 ✓  
140 µl ✓  
94.6 µl ✓  
3.5 µl ✓

Cf=5

$\alpha^{32}$ P dATP 10 mCi/ml (ref 6-2-57)  
3000 Ci/mmol

1.5 µl

Tube # 1-4 5-8 9-12 13-16 17-20 21-24 25-28 29-32  
(1) (2) (3) (4) (5) (6) (7) (8)

[A] 98 µl  
2 µl TFI in epimut units  
(its ~ 3.5 units each)  
TFI LUISB 2  
+ Vent (5-16-95) 10 µl →  
0.09 units Vent/µl

2x 1x Cheung  
- (no enzyme)

0.18 units  
Vent in

TFI Epimut SB 2 →  
+ Vent 5-16-95

TFI Epimut enzyme 2  
+ Vent (5-16-95) 15 →

Vf ~ 100 µl

2 µl  
0.045 µl  
Vent diluted  
in 1x Cheung  
buffer

mistake : this is  
0.09 units / 100 µl  
be 0.18 - if this use  
per 50 µl PCR

2 µl of Vent  
2 µl of 2-  
in 86.9 µl  
1x Cheung

68°C  
start with addition of enzyme to preheated mix.  
remove 10 µl to 5 µl 0.2 M EDTA (spot 10 µl on GTC  
and 5 µl to 5 µl Kellgren solution with cold IAM  
(spot 2) on PET at 0, 5, 10, 15, 20 min.

resolve in 1 M LiCl

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5-31-95



g N .		cpm - background cpm ↑ specific activity		ave pmole turned over ave pmole incorporated ↑ should be constant		2 replicates of each time pt	
SAM		pmoles		% turnover			
CPM1		turnover					
1	5	885.00	ex. $\frac{885-543}{103.31} = 3.31$	0.042		result: 1) turnover began to reach a plateau by 10 min. we expected turnover to continue increasing over time after DNA synthesis stopped. TFI competes w/ Vent at nick	
2	10	1241.00	4.76				
3	15	1074.00	5.14	0.069			
4	20	1269.00	7.03	0.065			
5		984.00	4.27				
6		1332.00	7.64	0.062			
7		1678.00	11.0				
8		1590.00	10.1				
9		830.00	2.78				
10		1213.00	6.49	0.021			
11		1195.00	6.31			2) turnover by Vent alone in Cheng buffer is lower than expected. 25% turnover was observed in another experiment. 453 pmole in 20 min. 25% 1/25 sm by Vent. The high signal to noise level for the Vent samples makes it difficult to say what the turnover is. Turnover is higher when Vent is mixed w/ TFI. TFI creates mismatches that are targets for Vent exo. do: repeat w/ 1, 2, 3, 4, 5 min time points and more Vent enzyme in the Vent alone samples, use new NEI plates	
12		1460.00	8.88	0.066			
13		555.00	0.116	0.055			
14		1228.00	6.63				
15		1225.00	6.60	0.062			
16		1425.00	8.54				
17		764.00	2.74				
18		977.00	4.20				
CPM3				0.039			
19		1212.00	6.48	0.028			
20		1453.00	8.81	0.043			
21		895.00	3.41				
22		772.00	2.22	0.061			
23		1009.00	4.51				
24		1365.00	7.96				
25		746.00	1.96	0.70			
26		438.00	1.02 X	0.47			
27		757.00	2.07				
28		609.00	0.64	0.14			
29		412.00					
30		578.00	543				
31		488.00	background				
32		693.00	no enzyme				

specific activity: cpm of 2nd spot of mix A 36438  $\bar{x} = 41,324$  cpm  
2 replicates  $\leftarrow + 40210$

$$\left( \frac{100 \mu\text{L rxn}}{2 \mu\text{L spot}} \right) \left( 41324 \text{ cpm} \right) = 103.31 \frac{\text{cpm}}{\text{pmole (nt) DNA}}$$

$$\left( 5000 \text{ pmole}_{\text{int}} \right) (4 \text{ bases})$$

50  $\mu\text{M}$  each dNTP in 100  $\mu\text{L}$  rxn  
50  $\mu\text{mole/L} \times 100 \times 10^{-6} \text{ L} = 0.005 \mu\text{mole} = 5 \text{ nmole} = 5000 \text{ pmole}$

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$$\frac{\text{sample cpm}}{\text{specific activity}} \times \frac{100 \mu\text{l rxn}}{10 \mu\text{l spot}} \times \frac{15}{10} \text{ dilution}$$

Incorporation

pmoles

1	49596.00	—	49596.00 × 15 = 7,201
2	74066.00	—	10,754
3	88521.00	—	12,853
4	95661.00	—	13,889
5	50395.00	—	7,317
6	69543.00	—	10,097
7	82738.00	—	12,013
8	93515.00	—	13,578
9	45114.00	—	4,550
10	64768.00	—	9,404
11	81250.00	—	11,797
12	96711.00	—	14,042
13	49095.00	—	7,128
14	71796.00	—	10,424
15	81335.00	—	11,809
16	95798.00	—	13,909
17	50290.00	—	7,302
18	70938.00	—	10,230
19	88754.00	—	12,887
20	98147.00	—	14,250
21	48881.00	—	7,097
22	85245.00	—	12,377
23	85694.00	—	12,442
24	91420.00	—	13,274
25	1932.00	—	281
26	2581.00	—	375
27	3000.00	—	436
28	3120.00	—	453
29	854.00	—	123
30	777.00	—	113
31	31183.00	—	26.6
32	32487.00	—	70.7
33	9.00	—	
34	6.00	—	

2ul mix A = 36438.00 -  $\bar{x}$  41,324 cpm  
 46210.00 for calculation of  
 specific activity

105 cpm/pmol

Synthesis ~~test~~ was almost complete by 10 min.  
 By 20 min. ~14 nmoles of the 20 nmoles  
 had been incorporated - hi, in the 1st

To Page N

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5-31-95

new dilution of rlog lot EKBT1  
to 5<sup>u</sup>/ml

Project No. \_\_\_\_\_

Exhibit L-100

Book No. \_\_\_\_\_

Appl. No. 09/558,421

43

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EKBT1 157.2 ml  
323 u/ml (see p91, 9)

Tag storage buffer 10 ml  
(Princl detergent)  $V_f = 10^{.157}$  ml (cf = 5<sup>u</sup>/λ)

mix end over end 1 hour

storage buffer is from 12-7-94  
with Princel Detergents

To Page No. \_\_\_\_\_

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Project N

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TITLE

primer degradation using TFI//  
(can see P 14, reaction # 11)

44

From Page N

(1) (2) (3) (4) (5) (6)

5 X Chemg  
(no JNT<sub>2</sub>)20  $\mu$ l —————→320 "33 mer correct"  
same as (P 12 and 14)

4 —————→

5  $\mu$ MTFI/Vent 5-12-95  
CTI SB

10

TFI/Vent 5-13-95  
Epiarth SB

10

(0.9 units vent  
in 10  $\mu$ l res)TFI/Vent 5-14-95  
Epiarth TFI

10

TFI LTI 4.33  $\mu$ l (P.8)

10

Vent .09  $\mu$ l

10

lot #17 (original 2-24-95)

LTI SB P.6 (same stock as in TFI P.8)

10

H<sub>2</sub>O

66

V<sub>f</sub> = 100  $\mu$ l\* 2  $\mu$ l Vent.  
diluted into  
CTI Tag SB  
2  $\mu$ l Vent  
42.4  $\mu$ l Tag SB  
44.4  $\mu$ l

68°C.

remove 10  $\mu$ l to 5  $\mu$ l appt res  
stop vol at 0, 5, 10, 20, 40, 60, 80, 100 madd enzyme on ice → take 0 time point,  
start timing when thin walled tube  
put in prewarmed 9600 at 68°C

1.6 % PAGE

~ 44 watts (Volts range from 1600  
get ~ 12 cm/hr for BPB

To Page N

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check  $^{32}P$  33mer (P44) on PEG

Project No. \_\_\_\_\_

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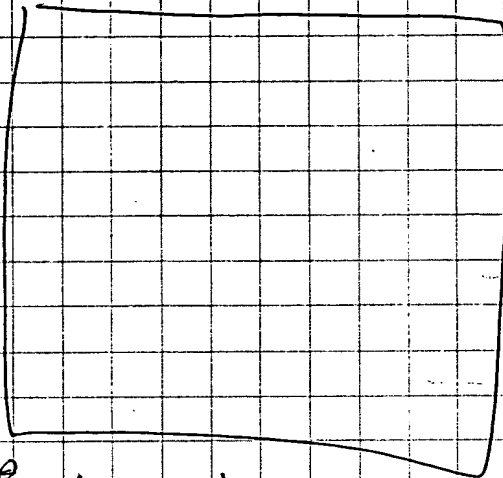
45

Page No. \_\_\_\_\_

$^{32}P$ -33mer is  $\sim 10 \times 10^6$  cpm/ $\mu$ l  
↓ dilute  $1/1000$

$1.5 \times 10^{-6}$  M  
~~65  $\mu$ mol nt~~ of 23mer  $\sim$  20 ~~75~~ mM nt  
5  $\mu$ l H<sub>2</sub>O  
5  $\mu$ l

10 mM dATP stock



100 mM stock  
→ 1:5 dilution  
10  $\mu$ l stock, 40  $\mu$ l H<sub>2</sub>O  
TE

1 M ATP  
1 M dATP

1 M 23mer  
cold

33mer  
 $1/1000$

RT 2.0 min

RT 25.0 min

3.0

5.0

1

2

3

2

2

2

2

2

To Page No. \_\_\_\_\_

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Result:

1. ice does not shut down 3' exo. start with Mg. left (?)
2. there is very rapid loss of first 1-10 nts, then very slow degradation. is next time, need 'less engaged' and/or shorter time points
3. No apparent ends of 5' exo activity for TFI alone as seen on P14. Here its 5' TFI (4.33 u/l) per 5' compared to 2 u/l on P14 but still should see plenty of loss of full length here based on rate seen on P14. also was P14 but on P14 try ± JNTPs  
also P14 has JNTPs present but not here. Maybe some kind of primer extension involved in loss of full length primers - is extension to many long contributes to apparent loss of primer or to production of a 5' exo target - could be primer primer extension or hairpin within 33 mer.

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D. Polay

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6/9/95

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Rec rd d by

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To Pag No

# Primer degradation by TFI/Vent

Project N \_\_\_\_\_

Exhibit L-102

Bo k N \_\_\_\_\_

Appl. No. 09/558,421

1

ag No. \_\_\_\_\_

'95-6/6/95

purpose: To measure 3'→5' exonuclease activity of TFI/Vent using the primer degradation assay.

background: An earlier trial of this experiment (NB 10 page) was done by removing aliquots of the rxn at time points 0, 5, 10, 20, 40, 60, 80, 100 min. The primer was degraded almost to the maximum amount by 10 min. Since we want to determine the 3'→5' exo activity rate, we need to find the linear range of the assay. This can be done by taking shorter time points or by taking a single time point on a series of enzyme dilutions (doubling [enzyme] should double extent of degradation in the linear range of the assay). We'll do this trial expt w/ just 1 enzyme sample - TFI/Vent in LTI SB - and 13 different dilutions. Once the linear range is found, we can repeat the exp. just on that range.

materials:  $^{32}\text{P}$  & ATP for end labeling primer  
primer = 33mer correct  
Taq dilution buffer - cc aliquot  
LTI storage buffer - RL aliquot  
TFI/Vent enzyme mix - from stability study  
9600 PCR machine & tubes  
5x Cheng buffer - cc aliquot  
PNK = T4 kinase & buffer - ~~SEL~~ LTI - new  
8% sequencing gel & buffer - LTI premade  
① stop buffer  
sterile  $\text{H}_2\text{O}$

To Page No. \_\_\_\_\_

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Polars

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6/9/95

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Recorded by

C. J. Conk

Date

6/8/95

From Page No. \_\_\_\_\_

## Procedure

4/5 ① end-label the 33-mer primer w/  $^{32}\text{P}$  & ATP = Kinase reaction  
reference = notebook 10 p 12

mix:	$\text{H}_2\text{O}$ sterile	25ul	✓	+ 25ul	added after 1st 5' con. were added
	5x Kinase buffer	12ul	✓	+ 12ul	
20.0ul	33mer constant	15ul	✓	+ 15ul	
AC4521	$^{32}\text{P}$ & ATP 10mCi/ml	5ul	✓	+ 5ul	
	PNK 1u/ul new FES419	3ul	✓	+ 3ul	
		60ul		120ul	

incubate  $37^\circ\text{C}$ , 30min ✓  
~  $55^\circ\text{C}$ , 5min ✓

store labeled DNA + unincorporated label at  $-20^\circ\text{C}$  - run some ok

4/5 ② make a ~~8~~ 8% denaturing-sequencing gel + buffer → both premade by

- 1, 75mL bottle of 8% mix (cold room) + 450ul  $^{32}\text{P}$  AP (made fresh)

0.0868g AP

363ul  $\text{H}_2\text{O}$  → 0.0868g  
0.868mL

- after pouring gel, shake up remaining gel mix so it can be used to fill leaks, ect.

- store gel upright, ON, at RT w/  $\text{H}_2\text{O}$ -soaked towels and saran ✓

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Paulyn C. Smith

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6/9/95

To Pag No



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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

4

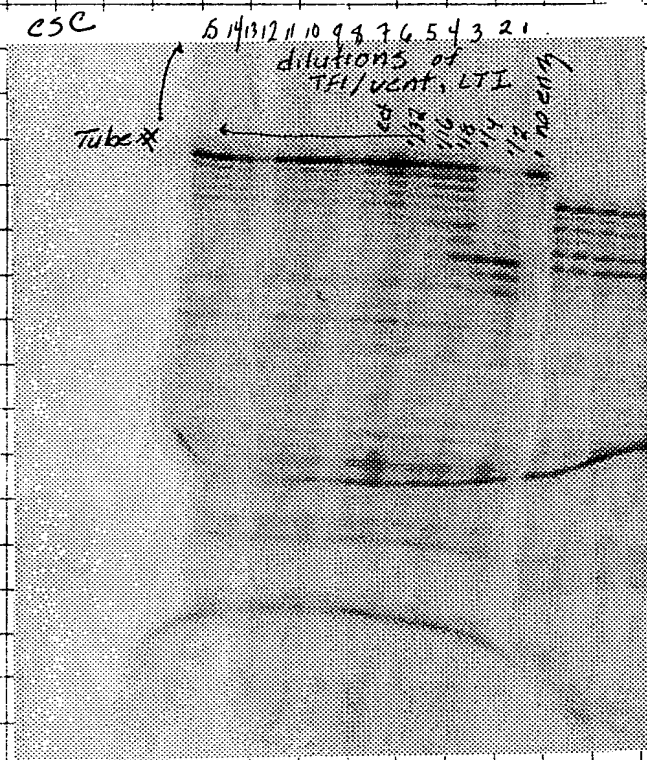
From Page No. \_\_\_\_\_

- B.P.D. ran down to bottom glass clamp on gel rig, ~1 hr, <sup>constant</sup> 1700 V, ~6.5W
- transfer to <sup>drain buffer</sup> Whatman paper
- cover w/ saran & cut to size
- dry - 2 pieces whatman under gel, saran over gel, dry ice in trap
- set vacuum & heat for 1 hr = 12:45-1:45 PM
- set in phosphorimager cassette - bottom to bottom w/ saran ~2 PM for ON exposure

Result: ON exposure on phosphorimager

csc

C:\DATA\CC.GEL 1995:06:07 07:57:48, Range = 0.11-10000.00 Counts, 0.50x



Conclusion: The 1/4, 1/8, 1/16 dilu  
 gave span the linear range  
 the primer degradation  
 Now, we'll do a cou  
 time pts of each  
 dilution to gather  
 better <sup>data</sup> from the lin  
 range. The data w/  
 used to show <sup>3-5</sup> exo ac  
 for stability study

To Page No

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6-9-95

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6/9/95

R c rded by

Paula Coult

Page N — 6/6/95

Purpose: To determine the relative mobility of the  $^{32}\text{P}$ -33mer correct primer on a PEI plate, developed in LiCl.

Background: Originally we wanted to determine the specific activity of the  $^{32}\text{P}$ -33mer primer that was used in the primer degradation assay w/ TFI/Vent (NB 10 p). However, we later decided that it is not important to find the specific activity since we can do a no enzy. control each time the assay is done. Now we want to determine the mobility because we observed that the cold oligo did not run as expected on the PEI plate, and we just are curious about how where the oligo ran.

Materials: cold 33-mer correct  
 $^{32}\text{P}$  33mer correct - labeled on 6/9/95

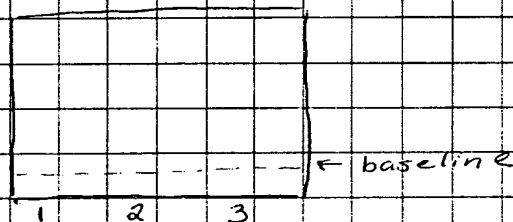
ATP

PEI plate

1M LiCl

scint vials = cocktail

Procedure: > spot on PEI plate →



20mM ATP 2ul

2ul

20mM 33mer

2ul 2ul

 $^{32}\text{P}$  33mer

2ul

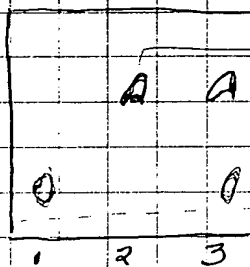
> set plate on developing chamber w/ 1M LiCl ~1hr - ran 1/2 way

> circle control spots (ATP + cold 33mer) in lane 3 under UV light → sketch of how plate looked

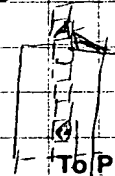
8 ATP  $^{32}\text{P}$   
 ADP  
 $^{32}\text{P}$ -33mer\*  
 cold 33mer  
 PP.  $^{32}\text{P}$ \*

cut

> lane 3 into 8 pieces and count in scintillation counter



expected to stay at base, but ran near the moving front



To Page No. \_\_\_\_\_

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6/9/95

Invented by

6-12-95

Date

6/9/95

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Cowan &amp; Co.

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Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

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CSC

Result:

PAGE:

USER: 1 ID:32P 1.0 CPM PRESET TIME: 1.00 TUE 06 JUN 1995 15:36  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N  
 H#: 0 AQC:N BCF:N RCM:N  
 CHANNEL 1-LL: 0 UL:1000 2SIGMA: 0.05 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:  
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000  
 HALF LIFE(DAYS):N

SAM	CPM1	TIME	EF
1	111.00 - baseline	1.00	
2	307.00 - area around where cold ATP	1.00	
3	136.00 standard ran	1.00	
4	117.00	1.00	
5	60.00	1.00	
6	215.00 - area around where cold 33mer primer ran	1.00	⇒ primer runs like dAMP
7	27.00 - near solvent front	1.00	

The labeled 33-mer primer ran like the dAMP runs on a PEI plate, up near the solvent front, not at the origin

This result was expected from the information we heard from the chemistry group: DNA stays at the origin because of its large size

oligonucleotides run like dAMP because they have same charge:mass ratio as dAMP

To Page 1

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6/9/95

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6/9/95

Paulen P. P. P.

TFI/Vent primer degradation assay - time course on serial dilutions for a time point of stability study

ig N . -  
 To measure 3'-5' exonuclease activity of TFI/Vent mixes in 3 buffers at the zero time point of stability study

Background: Linear range of assay determined in previous expt, NB11 page 1 - dilutions 1/4, 1/8, 1/16 looked good  
 - linear range  $\hookrightarrow 0.045$  units  
 - now do time course of these dilutions

1/4 dilution  $\rightarrow \frac{0.09 \mu\text{L}}{4} \times 2 \mu\text{L} = 0.045$  units 1/8 dil  $\rightarrow 0.0225$  units 1/16  $\rightarrow 0.0113$  units in 100L reactions

Materials:  $^{32}\text{P}$  33mer correct - labeled on 6/5/95  
 TFI/Vent in LTI, epicenter SB, epicenter TFI  
 8% gel  
 mix A

x Vent dilution 2000 u/mL  
 2 uL Vent Lot 17 2/24/95  
 44.4 uL TAE 313  
 44.4 uL at 0.09 u/uL  
 22.2 x dil

Procedure:

1) make mix A, enough for 14 rxns - 90 uL per rxn  
 per rxn  $5.6 \mu\text{L} \times 14 = 78.4 \mu\text{L}$   $20 \mu\text{L} \times 14 = 280 \mu\text{L}$  5x Cheng  
 $4 \mu\text{L} \times 14 = 56 \mu\text{L}$   $32\text{P}$  33mer sum stock  
 $66 \mu\text{L} \times 14 = 924 \mu\text{L}$   $\text{H}_2\text{O}$   
 $90 \times 14 = 1260 \mu\text{L}$  mix A

2) each enzyme/buffer mix

stop tubes	1-4	5-8	9-12	13-16
37-40	37-40	37-40	37-40	37-40
29-32	29-32	29-32	29-32	29-32
13-16	13-16	13-16	13-16	13-16
5-8	5-8	5-8	5-8	5-8

3) mix A — 90 uL 90 uL 90 uL  
 $\text{H}_2\text{O}$  — 8 6 4  
 undiluted enzyme (0.09 u/uL) 2 4 6

take 3, 6, 9, 12 min time points by removing 10 uL of rxn to 5 uL stop in small tubes, keep on ice

start rxns 1 min apart time on clock - 0 1 2 start

1 no enzyme control 30 uL 10 uL Cheng 2 uL  $^{32}\text{P}$  33mer 30 uL  $\text{H}_2\text{O}$  30 uL + 2.5 uL stop

3	4	5	stop
6	7	8	stop
9	10	11	stop
12	13	14	stop

To Page No. \_\_\_\_\_

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6/9/95

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6-12-95  
 Record d by

Date

6/19/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

10

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Note: samples Vent/Tf1 in LTI, 3 min 2nd > only 5th of can was stopped → load more 4.5 instead  
3 min 4th

4:50 PM - ~6:15 PM 1700V constant, -4.5W, gel was dried & put in P.I.  
 \* 39-40 may be underloaded due to problem expelling full vol. from  
 order: control, 1 → 48 where 1-12 are Tf1/Vent in LTI SB  
of samples on gel 13-24 are Tf1/Vent in epicenter SB  
 25-36 are Tf1/Vent in epicenter Tf1  
 37-48 are Vent alone.

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Repeat unit assay for TF1/V.  
incorp of P.30 for stability of

48

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Wms TF1/Vent mps of A J-  
with 5 duplicate dilutions  
for optimized signal/noise

Use 2  $\mu$ l of 1/250 dil for  
and linearity vs (units)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

TF1 Chezy unit  
assaying (same  
as P. 30)

47 —————→

TF1 4.33  $\mu$ l (P.8)  
1/250 dil \*

2 —————→

TF1/Vent LTISB

5-16-95 (Nin Guanid)  
1/250 notebook

2 —————→

TF1/Vent Epicure SB

5-16-95 1/250 dil

2 —————→

TF1/Vent (Epicure TF1)

5-16-95 1/250 dil

2 —————→

VF-50  $\mu$ l

74°C, 10' → 10  $\mu$ l 0.5M EDTA → spot 40  $\mu$ l on

\* all dilutions are done as 5 separate dilutions  
of 2  $\mu$ l Enzyme into 498  $\mu$ l Tag dilution buffer

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g N .\_\_\_\_\_

1	4163.00
2	4429.00
3	4636.00
4	4646.00
5	4349.00
6	4550.00
7	4529.00
8	4623.00
9	4350.00
10	4315.00
11	3995.00
12	4339.00
13	3732.00
14	4695.00
15	4428.00
16	3975.00
17	4584.00
18	4541.00
19	4297.00
20	4412.00
21	259.00
22	84613.00
23	87557.00

To Pag No. \_\_\_\_\_

ssed &amp; Understood by m ,

*Deena A. Baker*

Date

6/19/95

Inv nt d by

Record d by

Date

6-9-95



Test run of PEI plates - prior to turnover exp

ag N. \_\_\_\_\_

2/95

Purpose: To test how well a fresher batch of PEI plates can resolve dAMP from dATP and how tight/clean the spots are. This is being done prior to using this batch of plates for another TFI/Vent turnover experiment

Background: Last time the dAMP spot did not resolve well from the yellow "junk" that runs near the 1M LiCl solvent front, making it difficult to cut out + count just the dAMP (without Pi) for accurate turnover results. We'll try washing a different batch of plates - from Jesse - in dH<sub>2</sub>O + drying them 1st, before running samples and compare to unwashed plate. Also we see if running a whole plate gives better resolution than a 1/2 plate.

materials: PEI plates - from Jesse ← Macherey Nagel Polygram cell 30  
 Kill soln = ~20mM dATP  
 20mM dADP  
 20mM dAMP  
 100mM EDTA  
 1M LiCl - fresh, see recipe on next page  
 PEI/UV  
 Aldrich cat # 212,288-2

To Page No. \_\_\_\_\_

Sed & Understood by me, \_\_\_\_\_

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Recorded by \_\_\_\_\_

Date \_\_\_\_\_

6/12/95

Carolyn E. Smith

see P14 apparent exo present  
in PCR buffer Project No. \_\_\_\_\_  
50 P44, no exo in Book No. \_\_\_\_\_  
From Page No. \_\_\_\_\_

Repeat apparent exo result for TFI  
on P14 with different primer  
~~to home + hand lab~~

Exhibit L-107  
Appl. No. 09/558,421

① ② ③ ④ ⑤ ⑥ ⑦ ⑧

10x PCR buffer  
50 mM MgCl<sub>2</sub>

5 —————→  
10.5 μl —————→

5 ✓  
15 ✓

5x Cheung (no dNTPs)  
(see P200)

10 μl —————→ 10 ✓

32P 33mer correct (P51) 5 μm  
34P 23mer "AC" 5 μm  
32P 42mer "fidel" 5 μm  
see P12, 14 for method

2 2 2 2 ✓  
2 2 ✓  
2 2 ✓

Tag storage buffer

10 10

TFI 4.33 μl (P.8)

X10 —————→

H<sub>2</sub>O

31.5 —————→ 28 —————→ 31.5 28 ✓  
V<sub>P</sub> = 50 μl

74°C remove 10 μl to 5 μl cycle seq stop sol  
at 15 min 60 min

run on 8% PAGE

\* Zero time point:

1. mix buffer<sup>mix</sup>, 3<sup>2</sup>P primer (MgCl<sub>2</sub> if needed) and H<sub>2</sub>O. Volume =
2. remove 8 μl to 2 μl Tag storage buffer and 5 μl cycle seq. for 0 (no enzyme) time point.
3. now have 32 μl of reaction<sup>mix</sup> left. preheat to 74°C, add 8 μl TFI so V<sub>P</sub> = 40 μl again and remove 10 at 15 and 60 min to 5 μl cycle seq stop sol

T Page 1

Witnessed & Understood by me,

Date

Invented by

Date

D. Eugene Polansky

6/1/95

Record of by

6-13-95

32P

oligos (follow P12, 14 to 5 μM)

Proj ct N \_\_\_\_\_

Book N \_\_\_\_\_

51

Pag No. \_\_\_\_\_

5X kinase buffer  
"correct" 20 μM  
1379

[1]

[2]

[3]

4 μl

5 μl

✓

✓

mer AC(P1365)  
100 μM

1 μl

✓

← 23mer has termin  
A instead of G  
its called "AC"

mer "fidel" 6-13-95 old Temp  
100 μM  
ATP 1 μM (ref 6-16-95)  
VR  
H<sub>2</sub>O

This  
is different from  
Fidel Temp P14

1 μl

✓

2 μl

1 μl

8

12

12

✓

✓

← (5X less ATP  
than on P12)

$V_F = 20 \mu l$

37°C, 30', 5', 55°C

2 mer fidel

# 51351 CAC (012)

56.89 nmoles primer

56.89 μl H<sub>2</sub>O

F = 100 μM

To Pag No. \_\_\_\_\_

ss d & Und rstood by me,

Date

Invent d by

Date

Severina Polina

6

11/9/95

R corded by

6-13-95

Incorporation and turnover by Vent alone  
Time course on 0.1, 0.2, 0.3 units Vent

ag No. \_\_\_\_\_

purpose: To measure both incorporation and turnover by varying amounts of Vent over a 1 hr time course. These values will be compared to those of TFI/Vent mixes. Vent alone is a control for the stability assays of TFI/Vent mixes.

background: NB 10 p. 41, turnover was not above background for Vent alone when 0.09 units were used in a 20 min time course. <sup>This time well try more conc.</sup> However, Roger had observed turnover over background when 0.15 units were used 100ul rxn. Turnover is expected to increase linearly w/ time even after incorporation has stopped, if Vent can bind at the nick. New PET plates may give better results than last time.

materials: PET plates from Jesse, tested on NB 11, p. 15, Vent 2u/ul  
 activated DNA = gapped DNA made w/ DNase I, Kill 50(n = NB 11 p. 14)  
<sup>20mM dAM, DT</sup>  
<sup>100mM EDTA</sup>  
<sup>80mM dAM, DT</sup>  
<sup>100mM EDTA</sup>  
<sup>32P</sup> dATP - ref date 6/16/95  
 mix A: per 1, 100ul rxn: { 63.786 ul H<sub>2</sub>O  
 20ul 5x Cheng buffer  
 13.5ul activated DNA, 3.7 mg/mL, Cf = 0.5  
 0.5ul dATGC-TP, 10mM each, Cf = 50u  
 0.214 ul <sup>32P</sup> dATP 10mCi/ml

make enough mix A for today + the next expt - TFI/Vent turnover  
 16x 98ul/rxn = 1568ul

(for 16 reactions) A { 1020.6ul H<sub>2</sub>O as 1 mL + 20.6ul ✓  
 320ul 5x Cheng ✓  
 216ul DNA ✓  
 8ul dATGC-TP - BRL lot FBH001 ✓  
 3.424ul <sup>32P</sup> dATP  
 7.5 x 10<sup>7</sup> cpm total  
 4.7 x 10<sup>6</sup> cpm total  
 294 250 / 100ul reaction  
 7 x 10<sup>6</sup>  
 1569ul we 98ul/100ul rxn

Vent dilutions: - 2/4/95 Lot 17 sp(n) mix of Vent  
 LTISB = (100.5B)

1st dilute 5x Cheng → 10ul 5x Cheng + 90ul H<sub>2</sub>O - mix

① dilute Vent stock (2u/ul) to 0.15u/ul → 2ul Vent + 24.66ul 1x Cheng LTISB

② dilute ① to 0.1u/ul → 10ul ① + 5ul 1x Cheng LTISB

③ dilute ② to 0.05u/ul → 5ul ② + 5ul 1x Cheng LTISB

Read & Understood by me,

\_\_\_\_\_

Date

6/19/95

Invented by

\_\_\_\_\_

Recorded by

\_\_\_\_\_

Date

6/13/95

To Page No. \_\_\_\_\_

From Page No. \_\_\_\_\_

procedure: - deliver 10ul of kill soln to 1.5mL Cppendorfs x 1-  
- label tops 10' 20' 30' 40' 50' 60', set on ice until

rxn #

1

2

3

4

stop tube #

1- ~~6~~7- ~~12~~13- ~~18~~19- ~~24~~

mix A

98ul / rxn

prewarm to 68°C

0.15 u/l Vent

2ul

start rxns by adding eng., keep at 68°C in 9600

Lot # 17

opened 2-24-95

0.10 u/l Vent

0.05 u/l Vent

no eng

2ul

Thin in mix  
of vent as in  
TFI/Vent  
mix

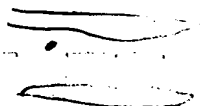
il thin  
0.2 u/l  
in 0.1  
Vent

2ul + change  
LTI SB  
= Tag SB

100ul

- remove 10ul rxn to stop tubes at each time pt (10', 20', 40', 50')
- spot 10ul / GFC + 2ul PEI
- L + 10ul of mix A x 3 (for determination of specific activity)

Test of old (Baker) PEI plates - 2ul, cold kill soln spotted



- old plate → dAMP runs w/  
solvent front junk

- new plate → dAMP runs ~ 1/2  
between solvent front & or

With ss d &amp; Und rst d by m

Deneen Pokrup

Dat

6/19/95

Inv nt d by

R cord d by  
Pawlyn P.omb

Dat

6/13/95

T Pag N

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Incorporation (pmoles)

20

From Page No. \_\_\_\_\_

$\left(\frac{\text{cpm}}{\text{specific activity}}\right) \left(\frac{100 \mu\text{l rxn}}{10 \mu\text{l spot}}\right) \left(\frac{20 \mu\text{l}}{10 \mu\text{l}}\right)$

0.3 mits

1.2 units

0.1 units

no eng.

55	21752.00 - 936 pmoles
56	40494.00 - 1742
57	50701.00 - 2745 2181
58	63810.00 - 2723 2745
59	63423.00 - 2723
60	61923.00 - 2663
61	12710.00 - 547
62	21727.00 - 934
63	32040.00 - 1378
64	39939.00 - 1718
65	43064.00 - 1852
66	51401.00 - 2211
67	9060.00 - 390
68	14810.00 - 637
69	19948.00 - 858
70	24421.00 - 1050
71	31940.00 - 1374
72	30490.00 - 1311
73	420.00
74	540.00
75	299.00 $\bar{x} = 348$
76	310.00 $n = 6$
77	197.00
78	323.00
79	923719.00 10ul spot of mix A
80	973931.00 $\bar{x} = 929,40.2$ for specific activity
81	890737.00

The <sup>observed</sup> specific activity of mix A is 2x higher than anticipated by the following calculation:

$$\frac{10 \mu\text{Li}}{\mu\text{L}} \times \frac{2.2 \times 10^6 \text{ cpm}}{\mu\text{Li}} = \frac{2.2 \times 10^7 \text{ cpm}}{\mu\text{L}} \times 3.4 \mu\text{L in mix A} = \frac{8.7 \times 10^7 \text{ cpm}}{1568 \mu\text{L A}} = 5.6 \times 10^4 \text{ cpm}$$

0.925 2 days to ref. date

$$\frac{5.6 \times 10^4 \text{ cpm}}{\mu\text{L A}} \times 10 \mu\text{L A spotted} = 5.6 \times 10^5 \text{ cpm expected}$$

9x10<sup>6</sup> cpm  
9.3x10<sup>5</sup> cpm observed

I don't know where the error came from, but the results should still be consistent within this experiment

T Pag No

Witness d & Und rsto d by m , <i>Deena C. Pokup</i>	Dat 6/19/95	Inv nted by <i>[Signature]</i> 6-19-95	Dat 6/14/95
R c rd d by <i>Paulum P. mdk</i>			

TfI/Vent in LTISB: turnover and incorporation  
5 replicates of 3 time points

Block No. \_\_\_\_\_

Fig N. \_\_\_\_\_

purpose: To <sup>more</sup> accurately determine turnover by TfI/Vent in LTISB  
- 5 replicates of the 5min and 10min <sup>15min</sup> time points, which are within linear range of the assay. This data will be used for the stability study.

ckground: also see NB 10 page for a time course of turnover, 2 replicates

materials: new PET plates  
mix A from 6/13/95  
TfI/Vent in LTISB  
Kill soln from NB 11 page 14 = 20mM each dA-MDP-P  
100mM EDTA

procedure: - deliver 20ul Kill soln to 1.5ml stop tubes 1-18 ✓

rxn x 5	1	2	3	4	5	6
top tubes	1-3	4-6	7-9	10-12	13-15	16-18

mix A 98ul ————— prewarm to 68°C —————>

TfI/Vent mix 2ul - add enzyme to start rxns  
LTISB 2ul

wick plate

2ul

2ul

2ul

2ul  
~~no enzyme control~~  
2ul LTISB = Tag SB

incubate at 68°C

100ul

> At 5, 10, 15 min remove 20ul rxn to the 20ul Kill soln in stop tubes, mix well & keep on ice

> spot 10ul/GEC filter (1-5) <sup>(1-5)</sup> spot rxn 6, no eng control 4x per time pt  
2ul/PET plate (1-5) <sup>(1-5)</sup> 27 x 2 = 54 = 12 spots

4 x 4 = 16

16-1, -2, -3, -4  
17-1, -2, -3, -4  
18-1, -2, -3, -4

To Page No. \_\_\_\_\_

Read & Understood by me,

Walter Polak

Date

6/14/95

Invent d by

Recorded by  
Carolyn Combs

6-15-95

Date

6/14/95





age N	Incorporation (pmol)
	(cpm/specific activity) $\left(\frac{100\mu\text{L rxn}}{10\mu\text{L spot}}\right) \left(\frac{40\mu\text{L}}{20\mu\text{L}}\right)$
5' 93072.00	93072/419 $\left(\frac{100}{10}\right) \left(\frac{40}{20}\right) = 4443$
10' 107957.00	-5153
15' 140583.00	-6710
5' 107888.00	-5150
10' 116159.00	-5545
15' 157153.00	-7501
5' 89224.00	-4259
10' 129878.00	-6199
15' 158185.00	-7551
5' 86678.00	-4137
10' 129770.00	-6194
15' 146342.00	-6985
5' 71757.00	-3425
10' 127388.00	-6081
15' 158825.00	-7581
no eng	285.00
5	355.00
no eng	291.00
10	300.00
no eng	310.00
15	262.00
background $\bar{x} = 301 \pm 31 \text{ cpm}$ 14.4 pmols $n = 6$	
839570.00	10ul mix A
831885.00	spotted 3X
840299.00	$\bar{x} = 837,251$

 $\bar{x} \pm 1SD$  Incorporation $n = 5$ 5'  $\Rightarrow 4283 \pm 620 \text{ pmol}$ 10'  $\Rightarrow 5834 \pm 467 \text{ pmol}$ 15'  $\Rightarrow 7266 \pm 395 \text{ pmol}$ 

$$\text{specific activity} = \frac{837,251 \text{ cpm} \times \frac{100\mu\text{L rxn}}{10\mu\text{L spot}}}{(5000 \text{ pmol}) 4} = 419.6 \text{ cpm/pmol (nt)}$$

To Page No. \_\_\_\_\_

is d &amp; Understood by me,

Zeeva Polansky

Date

6/19/95

Invented by

Record d by

C. Anthoni

Date

6/14/95

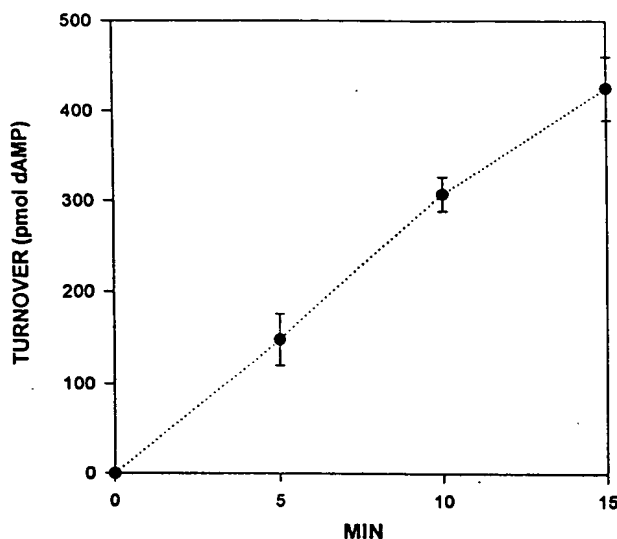
Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

24

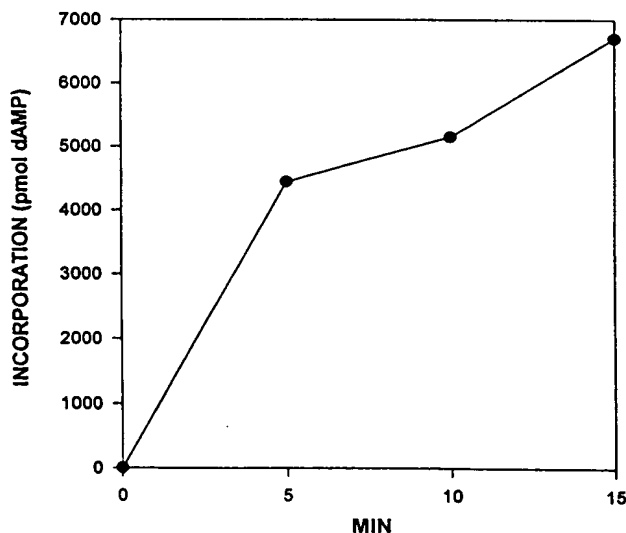
From Page No. \_\_\_\_\_

PROOFREADING: TFI/VENT



- 4/15/95  
cc
- Background was 118 pmd, so the best signal to noise occurs at 15 min (3.6x background). However, by 15 min, incorporation is slowing down as gaps are filled in. At 15 min some turnover is occurring at nicks - not a good model of a PCR reaction.
  - Trade off between good model and signal.
  - Linearity of the time points is good.
  - A 10% drop in activity would be detected by this assay, using 5 replicates.

POLYMERIZATION: TFI/VENT



- Incorporation falls off after 5 min, because gaps are filled by high TFI polymerization activity, after that, turnover occurs at nicks.
- % turnover increases because DNA synthesis is slowing down while turnover keeps going at the same rate.

T Page 1

Witnessed & Understood by me,

Date

Invented by

Date

*Doreen Polak*

6/26/95

Recorded by

*Paula Lomb*

6/25/95

ag N —	Turnover (pmoles) dAMP	% Turnover
$\frac{\text{cpm} - \text{background cpm}}{\text{specific activity}} \left( \frac{30}{10} \right) \left( \frac{100}{2} \right)$		$\left( \frac{\text{pmoles dAMP}}{\text{pmoles incorporation} + \text{dAT}} \right) \times 100$
CPM1 = dAMP + dATP = dAMP 1776.00 86470.00	ex. $\left( \frac{1776 - 807}{465} \right) \left( \frac{30}{10} \right) \left( \frac{100}{2} \right) = 208$	ex. $\frac{208}{208 + 936} \times 100 = 18.2$
> 20' 2994.00 - 86141.00	470	21.2
> 30' 4209.00 - 91512.00	732	25.1
> 40' 4983.00 - 85588.00	898	24.7
> 50' 6822.00 - 94359.00	1290	32.1
> 60' 7013.00 - 85869.00	1330	33.3
> 70' 1216.00 - 79679.00	88.0	13.9
> 80' 2179.00 - 83426.00	295	24.0
> 90' 2954.00 - 81631.00	462	25.0
> 100' 3716.00 - 83944.00	623	26.6
> 110' 4469.00 - 85258.00	788	29.8
> 120' 5283.00 - 87259.00	963	30.3
> 130' 1223.00 - 85430.00	89.5	18.7
> 140' 1807.00 - 90067.00	215	25.2
> 150' 2316.00 - 88894.00	325	27.5
> 160' 2953.00 - 84914.00	462	30.6
> 170' 3572.00 - 90268.00	595	30.2
> 180' 3815.00 - 92711.00	697	33.0
742.00 81173.00 749.00 87079.00 655.00 87371.00 880.00 86383.00 785.00 86929.00	$\bar{x}$ dAMP = 807 cpm is background = 174 pmoles	$\text{specific activity} = \frac{929,462 \text{ cpm} \times 100 \mu\text{l}}{10 \mu\text{l}}$ $\frac{(5000 \text{ pmole})(4)}{(4)} = 464.7 \text{ cpm}$ pmole nt DNA
CPM1		
978.00 - 90674.00		

To Page No. \_\_\_\_\_

Used & Understood by me,  _____	Date 6/19/95	Invent d by _____	Date 6/19/95
		Record d by _____	

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

30

From Page No. _____	Results:	Relative mobility, $RM = \frac{\text{distance from origin to center}}{\text{distance from origin to 50}}$			
4/20/95 CSC					
SAM	CPM1	distance (cm) from origin to center of rectangle	RM		distance from origin to 50
no enzyme, front at 9cm					
1	21456.00	0.65	0.17		
2	436.00	0.85 1.5	0.21		
3	217.00	1.65 1.9	0.26		
4	128.00	2.3	0.30		
5	105.00	2.7	0.41		
6	232.00	3.45	0.54		
7	73.00	4.9	0.66		
8	68.00	5.9	0.77		
9	78.00	6.9	0.91		
10	88.00	8.2			
60° vent, front at 9cm					
11	18209.00	same data as above			
12	572.00				
13	153.00				
14	87.00				
15	175.00				
16	1759.00				
17	119.00				
18	78.00				
19	151.00				
20	160.00				
60° vent, front at 13cm					
21	17132.00	same data as above			
22	42.00				
23	46.00				
24	99.00				
25	314.00				
26	2236.00				
27	60.00				
28	72.00				
29	98.00				
30	174.00				
no enzyme, front at 13cm					
31	21668.00	0.077			
32	250.00	2.25	0.17		
33	175.00	2.75	0.21		
34	112.00	3.25	0.25		
35	115.00	3.75	0.29		
36	201.00	5	0.38		
37	63.00	6.5	0.5		
38	71.00	8	0.62		
39	101.00	10	0.77		
40	100.00	12	0.92		
60° vent, front at 18cm					
41	17710.00	same			
42	326.00				
43	133.00				
44	125.00				
45	104.00				
46	1786.00				
90° vent, front at 13cm					
47	59.00				
48	56.00				
49	155.00				
50	121.00				
90° vent, front at 18cm					
51	16715.00	same			
52	400.00				
53	225.00				
54	82.00				
55	91.00				
56	2316.00				
57	76.00				
58	60.00				
59	100.00				
60	194.00				
60° vent, front at 18cm					
61	21188.00	1.25			
62	499.00	2.75			
63	195.00	3.25			
64	86.00	3.75			
65	83.00	4.25			
66	99.00	4.75			
67	81.00	5.25			
68	75.00	5.75			
69	139.00	7.25			
70	73.00	9.25			
71	66.00	11			
72	53.00	13			
73	130.00	16			
90° vent, front at 18cm					
74	19038.00	same			
75	333.00				
76	136.00				
77	79.00				
78	59.00				
79	90.00				
80	59.00				
81	73.00				
82	1811.00				
83	70.00				
84	50.00				
85	55.00				
86	262.00				
90° vent, front at 18cm					
87	18132.00	same			
88	449.00				
89	249.00				
90	72.00				
91	56.00				
92	76.00				
93	70.00				
94	66.00				
95	2231.00				
96	99.00				
97	45.00				
98	68.00				
99	234.00				

T Pag

Witness d & Und rst d by me,

Dat

Invent d by

Dat

6/26/95

6/20/95

R c rd d by

David Runk

Deanna Polansky

Primer degradation by TFI/Vent - Epicenter  
Time course & 5 replicates on 12% gel

Book No. \_\_\_\_\_

g No. \_\_\_\_\_

purpose: To measure 3'→5' exonuclease activity of TFI/Vent mix using the primer degradation assay. The data will be part of the stability study on the mix. The following changes to the assay will be made in hopes of obtaining more accurate data on the rate of primer degradation:

- 1) 12% gel will be used instead of an 8% gel - may give better peak resolution. Last time, peak shoulders and double peaks were a problem during quantitation.
- 2) 44mer<sup>Fidel</sup> will be used instead of 33mer - correct - the 33mer could form primer dimers &/or a hairpin that may have altered the degradation rate.
- 3) By doing 5 replicates, we can assess the accuracy of this assay as compared to the turnover assay.

Primer degradation by Vent alone will also be measured.

Background: Note - although we tested TFI/Vent mixes on p. 9 NB11, only the TFI purchased from Epicenter is TFI. LTI's TFI is really Tth. That is why we are not doing any stability tests on LTI's enzyme, until a new TFI alone is obtained.  
- the amount of enzyme and time course of this experiment are known to be in linear range of assay from the earlier expt on p. 9 NB11.

materials:  $\gamma^{32}P$ -end labeled 44-mer Fidel  
fresh mix A

12% denaturing sequencing gel

stop 50%<sup>12</sup>

TFI (Vent) mix made w/ TFI purchased from Epicenter  
Vent diluted w/ TFI Epicenter 50:50, Vent 10:17

recipe for 12% gel, 100mL:

48g urea  
30mL, 40% acrylamide:bis mix

10mL, 10x TBE

dissolve by stirring & low heat  
+ 600uL 10% AP (100ug/uL)

qs to 100mL w/ H<sub>2</sub>O - squirt bott.

30uL TEMED

100mL

To Page No. \_\_\_\_\_

Read & Understood by me,

slang

Date

6/26/95

Invented by

Recorded by

Carolyn Combs

Date

6/21/95

From Page No. \_\_\_\_\_

mix A: 90ul per rxn, enough for <sup>15</sup> rxns

$$6ul \times 9 = 54ul \text{ H}_2\text{O} \quad 99ul$$

$$20ul \times 9 = 180ul \quad 5x \text{ Cheng buffer } 300ul$$

$$4ul \times 9 = 36ul \quad 32P\text{-}44\text{mer Fidel, } 5uM \text{ stock } CF=200n$$

$$90 \times 9 = 810ul \quad 1350$$

procedure:1) end label the 44-mer Fidel primer - 70.3ul H<sub>2</sub>O

24ul 5x Kinase buffer

6ul 44mer Fidel, 100uM st

13.7ul <sup>32</sup>Pγ ATP, 10uCi/ul  
ref. date = 6.

6ul PNK 1u/ul

120ul

37°C, 30min

55°C, 5min

store at -20°C overnight

2) deliver 5ul stop soln to 9600 tubes ~~1-28~~ / label rxn tubes 1-  
make mix A

rxn #	1	2	3	4	5	6	7	8	9	10	11	12
H <sub>2</sub> O	8	8	8	8	8	8	8	8	8	8	8	8
mix A	90	90	90	90	90	90	90	90	90	90	90	90

preheat to 65°C in 9600, start by adding e

Cp. 50\*  
no enzyme  
8ul

2ul TFI / Vent - Epicenter 2ul Vent.

At each time pt remove 10ul rxn to the 5ul stop soln  
- heat 90°Cdo { no enzyme 0', 10' } replicas: 4', 6' at 70°C  
time course 2', 4', 6', 8', 10', 20' w/ TFI / Vent

T Page No.

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Date

Inv nt d by

Dat

D. Polans

6/26/95

R corded by

Dawson Pomb

4/21/95.

age N \_\_\_\_\_

picenter SB for no eng control: final 100mM NaCl, add solid to LTT SB  
and to dilute vent with.

final 50mM Tris

now at 20mM

5 mL Taq SB = LTT SB ✓

91.4 μL 1M Tris 7.5 - pre-made by LTT ✓

62.5 μL 1M Tris-HCl ✓ → 157.649/mole

153.5 μL glycerol ✓

0.031 g NaCl (58.449/m) ✓

$$\frac{1 \text{ mole}}{1} \times 10 \times 10^{-3} \text{ L} = 0.01 \text{ mole}$$

$$\frac{g}{157.649/\text{m}} = 0.01 \text{ mole}$$

$$= \frac{1.5764 \text{ g Tris-HCl}}{+ 10 \text{ mL dH}_2\text{O}}$$

Vent dilution in Epicenter storage buffer = 22.2x dilution to 0.094 μL  
2 μL Vent stock (2 μL/μL) w/ p2 pipetman → 1<sup>st</sup> spin down + vortex Vent stock  
+ 42.4 μL Epicenter storage w/ p200 pipetman  
44.4 μL vortex to mix

samples were heated to 90°C, 5' in 9600 prior to loading.

sample #'s

1 = no eng 0 min

2 = no eng 10 min

3 = TFI/Vent 2' min - not preheated = may be off

4 = " 4' 21 4 &gt; 2

5 = " 6' 22 6 &gt; 2

6 = " 8' 23 4 &gt; 3

7 = " 10' 24 6 &gt; 3

8 = " 20' 25 7 &gt; 4

9 (1) TFI/Vent 4' 26 4 &gt; 1

10 " 6' 27 4 &gt; 5

11 (2) 4' 28 6 &gt; 5

12 6' 29 6 &gt; 5

13 4' 30 6 &gt; 5

14 6' 31 6 &gt; 5

15 4' 32 6 &gt; 5

16 6' 33 6 &gt; 5

17 4' 34 6 &gt; 5

18 6' 35 6 &gt; 5

19 Vent 4' 36 6 &gt; 1

20 6' 37 6 &gt; 1

2<sup>nd</sup> load 1, 3 - 28, 12  
2, 3 - 28, 8  
1<sup>st</sup> load 1, 2 - 28, 2  
1, 2 - 28, 2 no times course

To Page No. \_\_\_\_\_

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Solamp

Date

6/26/95

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Recorded by  
Paula Corns

Dat

6/21/95

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TITLE \_\_\_\_\_

From Page N. \_\_\_\_\_

sample #

1<sup>st</sup> loading: 1, 9-28, 2 (no time course) ~3:15<sup>pm</sup>, rate  $\frac{0.7 \text{ cm}}{8 \text{ min}} \approx 0.1 \text{ cm/min}$

2<sup>nd</sup> loading 2, 3-28 w/ time course ~5:15<sup>pm</sup>, run until  $\beta$  reaches bottom. At this time ~10 bases will have run. This will be our whole gel loading to see the whole gel most of the products

Gel was run at 1700V constants for a total of 5.5 hrs.

T Pag N.

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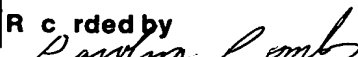


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6/21/95



From Page No. \_\_\_\_\_

PCR mix:

LTI 10x PCR buffer  
 50 mM MgCl<sub>2</sub>  
 4 dNTPs, 10 mM each  
 M13 RF 1 pg/ $\mu$ l  $\blacktriangle$  see page 42 for dilution  
 M13-6301 (anchor), 20  $\mu$ M  
 H<sub>2</sub>O

(40 Rxns)

400  $\mu$ l  
 120  $\mu$ l (1.5 M)  
 80  $\mu$ l (200)  
 40  $\mu$ l (10)  
 80  
 3160  
 3880  $\mu$ l H<sub>2</sub>O (400 primers)

(18 Rxns)

\* (in falcon tube)  
 H<sub>2</sub>O  
 rTag 5  $\mu$ l/ $\mu$ l  
 Tne 3.6  $\mu$ l/ $\mu$ l 5  $\mu$ l/ $\mu$ l

[1]

1746  $\mu$ l

18

1764  $\mu$ l

[2]

1746  $\mu$ l15.5  $\mu$ l (5  $\mu$ M / 100  $\mu$ l)2.5  $\mu$ l 18  $\mu$ l

1764

[1]

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16  
 98  $\mu$ l  $\longrightarrow$

[2]

98  $\mu$ l  $\longrightarrow$

ul 20  $\mu$ M primer see page 42-43

M136681

2

2

7069

2

2

407

2

2

806

2

2

1491

2

2

2506

2

2

3972

2

2

5464

2

2

100  $\mu$ l

make 2 sets of

1-16 gets elongation

1'-16' gets elongation

RE//

1 pg M13 / 100  $\mu$ l=  $3 \times 10^{15}$  mole nt=  $2 \times 10^{19}$  ~~moles~~ mo(2  $\times 10^{19}$ ) (6.0  $\times 10^{23}$  molecules)= 125,000 molecules  
arches

94°C, 1 min  $\longrightarrow$  30 cycles: 94°C 15 sec, 53°C 30 sec,  
 elongation is 6 min for PCR 1-16 and 2 min for 1'-16'  $\longrightarrow$  cont. on,

With ss d &amp; Und rsto d by m,

Dat

6/26/95

Inv nt d by

R cord d by

Dat

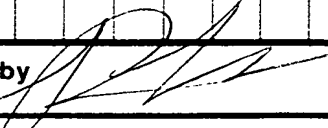
6-23-95

T Pag 1

The ad Tag with Cheng buffer  
for gap DH P

Project No. \_\_\_\_\_  
Book No. \_\_\_\_\_

ig N	1	2	3	4
5 x Cheng buffer (w/ dNTPs) (see P20, 10)	10	10	10	10
10 mM dNTPs	1	1	1	1
Human spleen genomic DNA 80 ng/μl	1.25	—	—	—
2112 12 μM	1	—	1	—
2113 10 μM	1	—	1	—
5' UTP Tag	0.5	0.5		
The 5' UTP H <sub>2</sub> O	35.25 50 μl	33.25	0.5 35.25	0.5 33.25

Designed & Understood by me, J. B. Olamp	Date 6/26/95	Invented by 	Date 6-23-95
		Recorded by	

To Page No. \_\_\_\_\_

19 N .

SigmaPlot regression lines:

1) for Proofreading: TFI/Vent the slope =  $28.676 \frac{\text{pmole}}{\text{min}}$ ,  $r^2 = 0.978$

2) for Polymerization: TFI/Vent the slope =  $417 \frac{\text{pmole}}{\text{min}}$ ,  $r^2 = 0.873$

Units of TFI/Vent proofreading:  $28.68 \frac{\text{pmole}}{\text{min}} \times \frac{30 \text{ min}}{10,000 \text{ pmole}/\mu\text{l}} = 0.043 \mu\text{l}$

Units of TFI/Vent polymerization:  $417 \frac{\text{pmole}}{\text{min}} \times \frac{30 \text{ min}}{10,000} / 2 = 0.63 \mu\text{l}$

expect:  $0.045 \mu\text{l}$

### conclusions

1) The turnover assay can detect a 10% loss of 3' exo activity in TFI/Vent mixes. By repeating the assay more frequently and/or with more replicates the error may decrease so that a 5% loss of activity could be detected.

2) Early time points, before 10 min, reflect turnover during DNA synthesis - the best model of PCR. Later time points reflect turnover during DNA synthesis plus turnover at DNA nicks - not such a good model of PCR. However, the later time points give better data because the signal to noise ratio is higher (3.6x versus ~1.3x early on). Both all 3 time points should probably be done during the stability study.

3) Turnover by TFI/Vent mix is about 3x higher than by Vent alone. This result was observed in an earlier experiment too. TFI may create more mismatches for Vent to turnover than when no TFI is present.

↑  
not true. exo in TFI/Vent is only ~ 2x higher than for vent alone (see mismatch on P 40, 10). In this experiment (P 17, 11) the Vent alone of 2  $\mu\text{l}$  of  $0.1 \mu\text{l}/\mu\text{l}$  is the one we should compare to the TFI/Vent mix.

To Page No. \_\_\_\_\_

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Ernest C. Polansky

Date

6/26/95

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Recorded by

Lawyer Combs

Date

6/24/95

PLATES

Both the dATP + dADP peak and dAMP peak become more spread out in the PEI plates as the solvent front runs further. The distance between the two peaks becomes greater as the solvent front runs further: ~0.5cm vs 2cm

Conclusion: For best resolution of dAMP from dATP + dADP on PEI plates, run the LiCl solvent front to the top of the plate, ~16 cm from origin.

To Page No. \_\_\_\_\_

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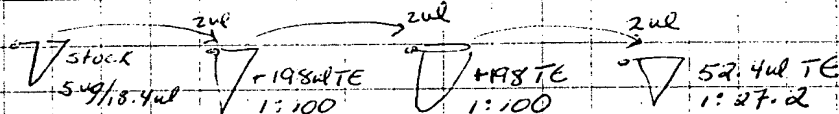
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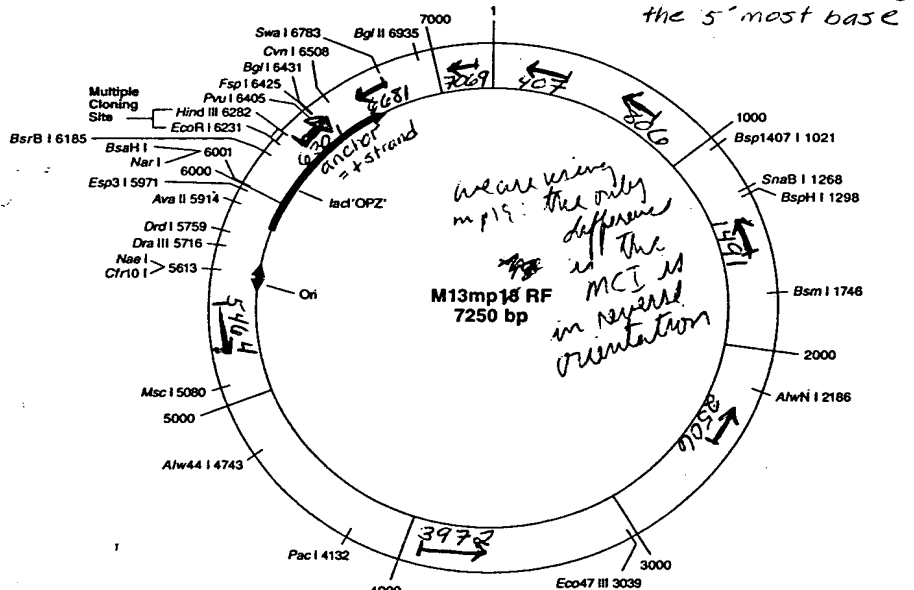
TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

Dilution of M13 RF to 1 pg/ul in TE: stock lot CN4132, 5 ug/18.4 ul  
do serial dilutions to  $1.272 \times 10^5$



6/20/95



number indicates the 5' most base

M13mp19 prime

→ 20mers

→ 9G+C

→ 11A-T

→  $T_m = \sim 58^\circ\text{C}$ 

primer name

PCR product length (bases)

sequence

M13-6301 anchor (= + strand)

5' GTTTTACAAC

M13-6681 (= - strand)

380

5' TTCC TGTAGCCAGCTTTC

M13-7069 "

768

5' ATG CCTGAGTAATGTGTAGG

M13-407 "

1356

5' GAAGCAAAGCGGATTGCA

M13-806 "

1755

5' TTA TACCACTCAGGACGT

M13-1491 "

2440

5' AGCTTGATACCGATAGTTGC

M13-2506 "

3455

5' CGACAG AATCAAGTTTGCC

M13-3972 "

4921

5' AATCGCAAGACAAAGAACG

M13-5464 "

6413

5' GTATAACGTGCTTTCTCC

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T Pag N

g N .

separation of primer stocks and dilutions:

Primers were made by Gibco BRL Custom Primers order# 510790A

Each primer was ~~that~~ resuspended in sterile dH<sub>2</sub>O at a CF=100µM  
- spun down, H<sub>2</sub>O added, 2min RT, vortex, invert

primer	nmols/tube	volume of dH <sub>2</sub> O added to make CF=100µM
M13-6301 anchor	54.7	547 µL
M13-6681	44.09	440.9
M13-7069	<del>39.14</del> 57.29	<del>391.4</del> 572.9
M13-407	39.14	391.4
M13-806	51.92	519.2
M13-1491	69.49	694.9
M13-2506	66.34	663.4
M13-3972	34.68	346.8
M13-5464	42.45	424.5

Each 20µM aliquots of each primer were made from the 100µM stocks:

⇒ 1:5 dilution, 40µL of 100µM primer stock

+ 160µL dH<sub>2</sub>O-sterile

200µL for each primer except M13-6301 anchor

1:5 dilution of M13-6301 anchor, 200µL of 100µM stock

+ 800µL dH<sub>2</sub>O-sterile

1 mL

inuation of PCR expt from p 40

10µL of 1Kb ladder and 10-18µL of PCR products were run on a 1% TAE agarose gel at 190V (~180mA, 40W)

% gel recipe: 220mL 1x TAE

2.2g agarose

wt = 435.6g, boil, reweigh & add H<sub>2</sub>O back to orig. wt

stir & cool

add 15µL, 10<sup>mg</sup>/mL CTBr & pour into rig. wt quarters

buffer: 2L 1x TAE

2170µL CTBr at each end

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Potemp

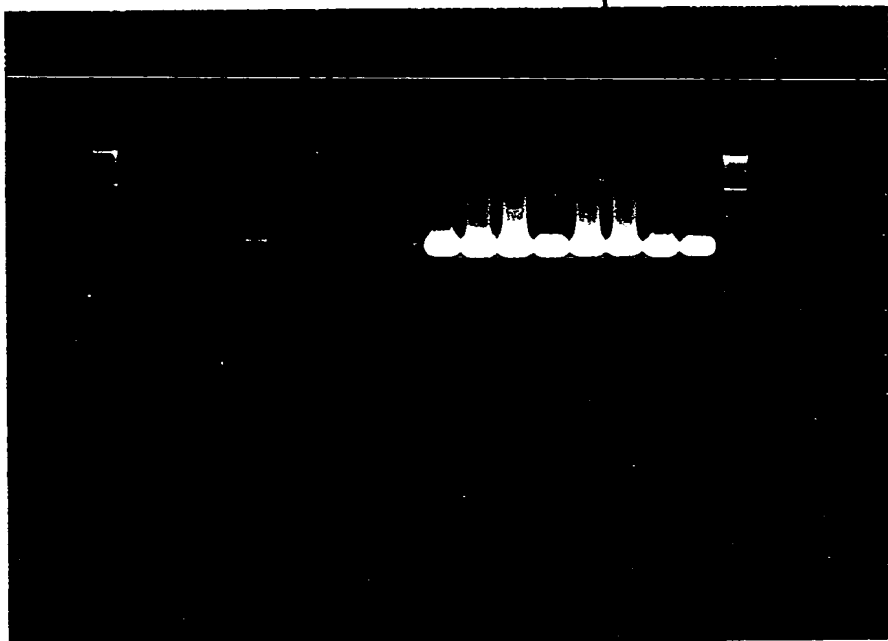
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6/26/95

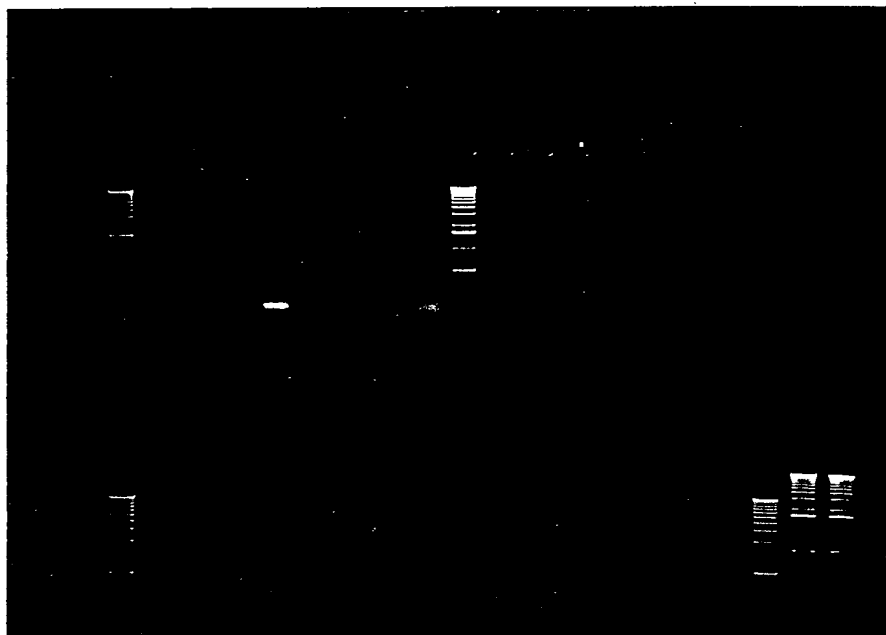
Carolyn Lomb

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taped into  
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> Since 10ul of the rTa  
PCR rxns contained suc.  
low level of product,  
tried loading 15ul in or.  
to be able to visualize  
products better

> No specific products  
seen in the Tne lat

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> note false product  
1491 primer  
- only 1 band of 2.  
was expected  
- instead there are 2  
~ 0.5-1 Kb and 2-3

> Note false product  
m13 2506 primer -  
should be ~3.5 Kb  
> also a short, false  
product w/ m13-5464

T Pag

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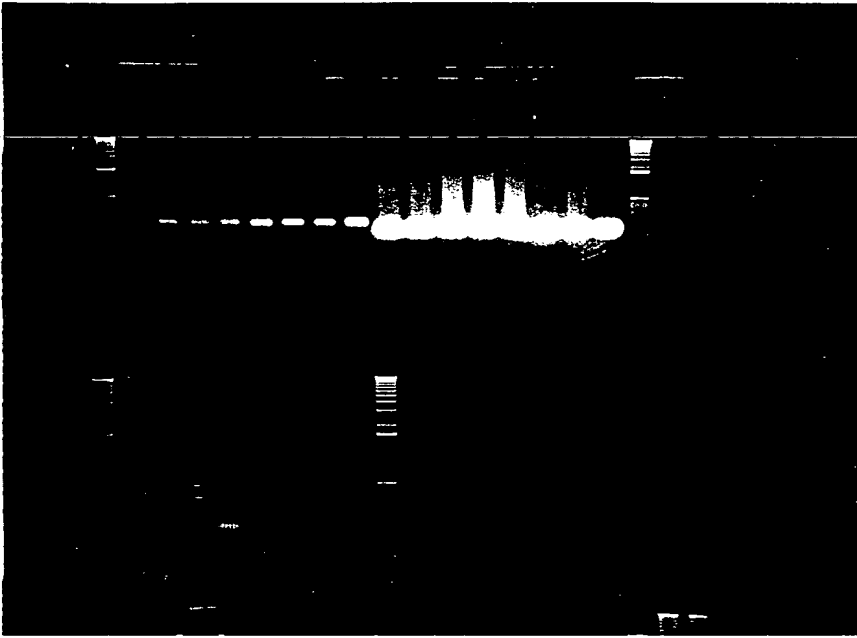
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David Lumb

Date

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ig N \_\_\_\_\_



> 6' extension time did not  
give more product. - worse  
yield than with 2' extension

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cc book 6/30/95

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*JS*

Date

6/30/95

Invented by

Recorded by

*Caulyn Comb*

Date

6/26/95



M13 PCR system: optimizing annealing temperature

ag N \_\_\_\_\_

[A]

(2 Runs)

LTI 10X ~~25~~ PCR buffer

120 ✓

50 mM MgCl<sub>2</sub>

36 ✓

4 dNTPs 10 mM each

24 ✓

M13 RF 1 pg/μl (lot # CN4132)

12 ✓

M13 6301 "Anchor" 20 μM

24 ✓

rTag 5 μl/μl

12 ✓

H<sub>2</sub>O

947 ✓

1.176 mL

195

1<sup>st</sup>51, 49, 47°C annealing temp w/ 1<sup>st</sup> 3 oligos program 19, 94°C

18, 94, 15"

1 2 3 4 5 6 7 8 9

51, 3"

70, 2"

[A] 97 μl →

3681	2		2		2		✓
3706	2		2		2		
3407	2		2		2		

V<sub>f</sub> = 100 μl

14	9600	51°C	annealing temp	1-3
15		49°C		4-6
16		47°C		7-9

2% gel: 2.64g agarose + 220mL

220mL

100k  
10kb  
ladder100k  
10kb  
ladder

(3.36g agarose + 280mL TAE wt = 480.3g)

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J. O. Long

Date

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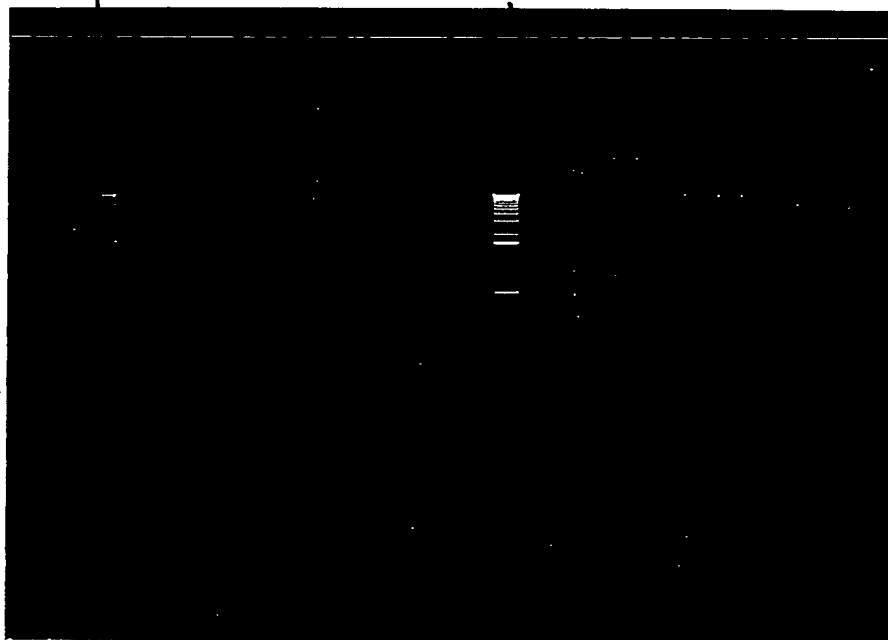
Cindy Pomeroy

Date

6/24/95

From Page No. \_\_\_\_\_

Result:



6/26/95 cc

primer combo	expected product length (bp)	observed product length (bp)
anchor + 6681	380	✓ looks like 380 relative
anchor + 7069	768	✓ 768 "
anchor + 8407	1356	✓ 1356

> specific products of the right length were made, but the yield was still v. low. Cole thought lowering the annealing temp might result in a higher yield. It did not. The lanes on the right side of the gel just look a bit darker because the light box is brighter on that side (note how the 10kb ladder looks more intense on right side even though 10ul was load on left & right sides. Also note

> Next we'll try to increase the yield by using denaturation time (from 15" to 30" - Veri's suggestion), [Taq], Δ cycle & [primer] - in c the anchor primer has a hard time annealing due to 2° struc. If it does, then lowering annealing temp would exacerbate the problem.

T Page No.

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N. O. O. O.

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P. O. O. O.

6/26/95

Δ denaturation temp, Δ [Tag]  
 Δ cycle number, Δ [primer]

Project No. \_\_\_\_\_  
 Book No. \_\_\_\_\_

g No. \_\_\_\_\_

10x PCR buffer  
 50 mM MgCl<sub>2</sub>  
 4 dNTPs 10 mM  
 M13 RF (lot FAS701) 1 pg/μl  
 opened 6-26-95  
 H<sub>2</sub>O

[A]  
 100 ✓  
 30 ✓  
 20 ✓  
 10 ✓  
 740 ✓  
 900 μl

different lot than on p. 40  
 dilution of stock  
 370 μl stock → 2 μl → 2 μl → 10 μl  
 198 μl H<sub>2</sub>O 198 μl H<sub>2</sub>O 360 μl H<sub>2</sub>O  
 $\frac{1}{100} \times \frac{1}{100} \times \frac{1}{37}$   
 =  $3.7 \times 10^5$  fold dilution

#	1	2	3	4	5	6	7	P
	90 μl							
	5' 1' 4'				5' 1' 4'			
	4 P 4 P				4 P 4 P			

1:1 mix of anchor + 407  
 30 μl 20 μM stock anchor  
 30 μl 20 μM 407  
 60 μl, 10 μM each  
 (400 or 800 nM oligo each)

5' μl 1 1 2 2 1 1 2 2 ✓

74°C, 1' initial denaturation

15" denaturation  
 94°C  
 ↓  
 53°C, 30 sec  
 ↓  
 70°C 2 min  
 Lab 15, 9600

30 sec denaturation  
 94°C  
 11  
 11  
 Lab 16, 9600

30 and 40 cycles 3:15 PM  
 620 μl rxn 1-8  
 + 2.3 μl 10x LD  
 Freeze ON  
 left in PCR machine, at 4°C ON

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	Recorded by Carolyn Cont		To Page No. _____

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Results P. 49

From Page No. \_\_\_\_\_

Denaturation time

15 sec

30 sec

units Tag

5

10

5

10

primer (nm)

400

700

400

700

400

700

400

700

cycles

30 38

30 38

30 38

30 38

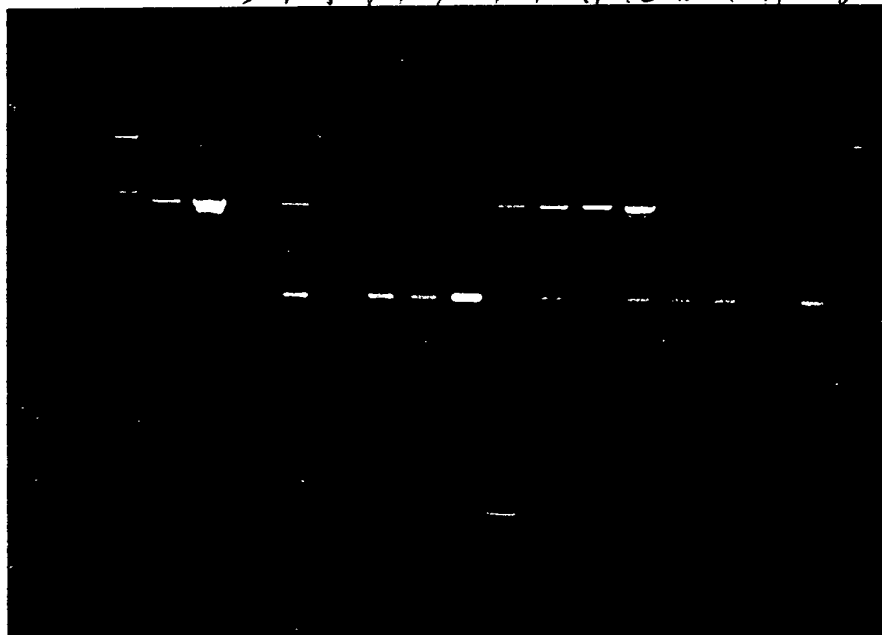
30 38

30 38

30 38

30 38

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



E 1356

← primer dimer

- . tube 1 compared to tube 3 on 49 shows new M13RE only gives little or no improvement to yield at 30 cycles
1. lower [primer] (400 nm) is best (tubes 1, 2 vs 3, 4) (when denaturation is for 30 sec, 400 vs 700 nm primer about equal)
  2. Lower Tag is best: only primer dimer made for 10 (# 5-8 and 13-16)
  3. 38 cycles made more product than 35 cycles (tube 2 vs 1)
  4. 30 sec denaturation gave less product than 15 sec.

T Pag N.

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Dat

6/30/95

Invent d by

R cord d by

Randy Roubin

Dat

6-26-95

Roubin

Page No. \_\_\_\_\_

Therefore:

keep 15 sec denaturation

Try even less primer (eg 100 200 300 400 nm)

Try even less tag (2, 3, 4, 5 min)

Try different cycle number (30 - 40)

Try M13 6301 (anchor) alone and with other primers  
with no target to look for primer dimerTry more M13 RF target  
eg. 0.1 pmol - 10 nmolTry R1 (or Bam, Hind III etc) "check buffer salt  
cut

PCR with no purification

Test all primers with best conditions

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

D. O. L. W.

Date

6/30/95

Invented by

Recorded by

C. O. L. W.

Date

6-26-95

Exhibit L-117  
 Appl. No. 09/558,421  
 Project No. \_\_\_\_\_  
 Book No. \_\_\_\_\_

52

1.1X PCT stability assays  
 TITLE 4°C storage (see P121, 9) -20°C and -70°C  
for freeze & thaw

From Page No. \_\_\_\_\_

	Rxn #	array	ul	
# 10 (P121, 9): no det	1-3	2	✓	This is 5 month point for 4°C studies
# 11 " 1.1X	4-6	3.64	✓	(same as P121, 9; 154, 9; 174, 9; 37, 10 = 0, 1, 2, 4 m)
amp Temp # 11: 1.1X	7-9	3.64	✓	(154, 9 is 0 time point, P 38, 10 is 1 month)
Tag # 125 del (same as P121, 9)	10-14	2	✓	
1.1X May 8, 1995	15-17	2	✓	called "new" on P 34, 10
1.1X field test	18-20	2	✓	called "old" P 34
1-27-95 -20°C	21-23	2	✓	Joel took aliquot from samples on 1-27-95 gave & freeze Thaw stored at -20°C, 5 months at with unknown effect from freeze/thaw
-20°C 5/24/95	24-26	2	✓	from 1.1X May 8, 1995 (compare to Rxn # 15-17 above) w/ month at -20°C with no extra freeze th
-70°C 5/24/95	27-29	2	✓	from 1.1X May 8, 1995 - its w/ month at -
* 10 freeze Thaw	30-32	2	✓	* used 1.1X May 8, 95 (above) in
20 freeze Thaw	33-35	2	✓	drying ETOH → 30°C bath no
30 freeze Thaw	36-38	2	✓	start with 60 µl and take out at 10, 20, 30 freeze Thaw
Fr 13-17-95 TNE				
P 25, 10				
1/700	39	2	✓	
1/700	40	2	✓	
1/700	41	2	✓	
TNE 5-7-95 (270.7 µl on P 25)				
1/8000	42	2	✓	
	43	2	✓	
	44	2	✓	
48 µl Tox unit assay mix (P120, 9) in each				
10' 74°C				
kill' with 10 µl 0.5M EDTA, spot 20 µl on 6-FC				

repeat of P25 for TNE did 5200 fractions look activity at 4°C

Witnessed & Understood by me,

*[Signature]*

Date

6/30/95

Invented by

*[Signature]*

Date

6-28-95

To Page!

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Appl. NO. 071550,421

B ok No.

Percent  
of zero time plus  
in P. 122/9

je N		u	u/1 relative to log (#10-74)		
1	1597.00				
2	1752.00				
3	1760.00				
4	7709.00				
5	6150.00	6760 ave	.030	94%	
6	6422.00				
7	4510.00				
8	5085.00		.025	72%	
9	5662.00				
10	8620.00				
11	9351.00	8931 CPM ave	.04 (by definition)		
12	8531.00				
13	8321.00				
14	9832.00				
15	5618.00				
16	5895.00	5132 ave	.025	10.9%	
17	5384.00				
18	5128.00				
19	5036.00	5215 ave	.023 u/1	10.9%	
20	5481.00				
21	3989.00				
22	4058.00	3673 ave	.023 u/1	54% recover	from .05 u/1 measure
23	2971.00				
24	5931.00				
25	5591.00	5921	1 month -20°C from 5-8-95 (1.1X #15-17 above)	101%	
26	6242.00				
27	5891.00				
28	5381.00	5712	from 1.1X of 5-8-95 (1.1X #15-17 above)	97%	
29	5865.00				
30	5644.00				
31	5407.00	5440	started with 1.1X (started only at 40°C)	96%	
32	5271.00				
33	5362.00	5405	(started only at 40°C) (1.1X #15-17 above)	100%	
34	5494.00				
35	5361.00				
36	5556.00	5438	conclude no loss of units for 30 freeze Thaws		
37	6159.00				
38	5200.00				
39	138.00				
40	287.00				
41	137.00				
42	1014.00				
43	960.00				
44	1092.00				
45	395.00				
46	110131.00				
47	110429.00				

The did at 4°C off 5200 col of 5-18-95  
got 7.7 u/1 on K25

ave - BK60 = 62.7 CPM = 27.3 p/mL [32.8 u/1] agrees with 36 u/1

of 21 may be wrong. The is stable until more data is available.

68.9 CPM/p/mL

ed & Understood by me,

Date

Invented by

Date - 30-95

Recorded by

(6-25-95)

May 8, 95

3 batch:

①  
17 sample  
original buff. formulation -

②  
Date  
field test

③  
(= "new" on P34, 10)  
500ul batch  
lot

created  
40C RT -200C 27/1

created

created

\* RT & NaN<sub>3</sub> \*

1.1x  
+ ~~original~~

\* 8x  
thaw \*

RT 40C \*

40C  
24/5/95  
add 1 g of 1-2 ul  
-20C -70C RT 40C \*

thaw  
make aliquots  
20 ul each  
as -20C  
only 1 or 2 freeze thaw

The unit array \*

notes from  
Joe Jolas

R Jolas  
6-27-95



Turnover by TFI/Vent - 7 replicates of \_\_\_\_\_ Book No. \_\_\_\_\_  
 Epicenter's TFI/Vent and LTI's TFI/Vent in Epicenters SB - zero time of for stability  
 ag N \_\_\_\_\_

pose: To establish the 3' exo activity of TFI/Vent by Turnover on  
 gapped DNA at time zero of stability study. 7 replicates  
 to reduce error

Background: The zero time point turnover assay has already been  
 for Vent alone and LTI's TFI/Vent in LTI SB  
 with 5 replicates NB11 page 17 and 21

pro to deliver 10ul stop & 10ul rxn-wipe tips

materials: mixA, enough for 40 rxns, each using 98ul of mixA ✓  
 for 23 = 1467ul H<sub>2</sub>O ✓  
~~40 x 63.786 = 2551.44ul H<sub>2</sub>O~~ → 4605x Cheng ✓  
~~40 x 20 = 800ul 5x Cheng~~ → 310ul act DNA  
~~40 x 13.5 = 540ul activated DNA~~ → 11.5<sup>1</sup> dATGC-TP  
~~40 x 0.5 = 20ul dATGC-TP, 10mM each~~ → 4.92ul <sup>32</sup>P dATP  
~~40 x 0.214 = 8.56ul <sup>32</sup>P dATP~~ → 2253.422 <sup>6/30</sup> Ame  
 3920ul

5	1-7	8-14	15-21	<del>22-28</del>	29-35
tube	1-21	<del>22-42</del>	43-63	<del>64-84</del>	85-91
	1-7	8-14	15-21	22-28	29-35
	1-21	43-63	85-91	15-21	43-49
x A	98ul	prewarm	to 68°C		

TFI/Vent (LTI SB)  
 2ul

1 Vent (epi SB) 2ul

1 Vent (epicenter TFI)  
 2ul

ent diluted (5-14.55)  
 2ul

used & Understood by me,

\_\_\_\_\_

Date

6/30/95

Invent d by

Recorded by  
 Carolyn Combs

Date

6-29-95

2ul LTI SB = Tag <sup>5/5</sup>  
 To Page No. \_\_\_\_\_

From Page No. \_\_\_\_\_

At 5, 10, 15 min remove 20ul rxn to 20ul stop soln (P14)  
 Spot 2ul on PEI plates  
 Spot 10ul on GFC filters (2 per rxn) + 2ul mix A 3x

tube #s

1 2 3  
 4 5 6  
 7 8 9  
 10 11 12  
 13 14 15  
 16 17 18  
 19 20 21

epi  
 epicenters Tfl enzyme + Vent in LTI SB  
 (=Tth?)

22 23 24  
 25 26 27  
 28 29 30  
 31 32 33  
 34 35 36  
 37 38 39  
 40 41 42

LTI's Tfl eng in ~~the~~ epicenters SB + Vent  
 =Tth

5 10 15  
 43-49

43-49 = no enzyme

GFC'S 1-6 = 2 replicates of epicenter eng + Vent  
 7-12 = 2 replicates of LTI's eng in epicenter SB  
 13 = no eng  
 16-18 = 2ul mix A

To Page 1

Witnessed &amp; Understood by me,

Date

Initiated by

Date

Recorded by

6/30/95

*[Signature]*  
*Paulson Pomb*

6-29-95

*[Signature]*  
*John Olson*

From Page No. — see. p. 51

purpose: The PCR worked well (gave a large amount of product when 38 cycles were done using 400nM primer

5 uL/100 uL Taq,  
15 sec denaturate

Now we'll try to get the same good plateau yield by optimizing [primer], [enzyme], checking for anchor problem [target] & linearizing target - using just 30 cycles

program 74 mix

expt 1.  $\Delta$  [primer] from 100, 200, 300, 400 nM w/ 5 uL Taq, 15" dena 30 cycles

make **A**:  
120 uL 10x LTI PCR buffer  
30 uL 50 mM MgCl<sub>2</sub>  
24 uL 10 mM 4 dNTP  
12 uL 1 pg/uL m13RF → dilute w/ TC  
888 uL H<sub>2</sub>O  
+ 1009 uL  
1000 uL

run	1	2	3	4
<b>A</b>	90	90	90	90
H <sub>2</sub> O	9	8	7	6
primer mix				

**A**:  
50 uL 10x PCR buffer  
15 uL 50 mM MgCl<sub>2</sub>  
10 uL 10 mM 4 dNTP  
5 uL 1 pg/uL m13RF  
395 uL H<sub>2</sub>O  
5 uL rTaq, 5 uL  
480 uL

2 uL of 370 ug stock + 198 uL TE - mix  
2 uL + 198 uL TE - mix  
10 uL + 360 uL TE ⇒ 1 pg/uL

make primer mix:  
6 uL 20 uM anchor primer  
6 uL 20 uM 407 primer  
12 uL 10 uM each

T Page 1

Withn ssed &amp; Und rst d by me,

00000000

Date

6/30/95

Invent d by

R corded by

Paul M. Lomb

Date

8-25-95

ig N	Tube	1	2	3	4
0		0	1	2	3
mer mix		4	3	2	1
9		96	96	96	96

Tag] from 1 to 7 units / 100ul rxn → 400nm primers

80ul	10x PCR buffer	✓	dilute rTaq 5x	✓
24ul	50mM MgCl <sub>2</sub>	✓	6ul rTaq	5 <sup>u</sup> /ul
16ul	10mM 4 dNTPs	✓	+ 24ul Tag	dilution buffer
8ul	1 pg/ul M13RF	✓	30ul	
16ul	20uM anchor primer	✓		
16ul	20uM 407 primer	✓		
584ul	H <sub>2</sub> O	✓		
744ul				

bx	5	6	7	8	9	10	11
3	93						✓
20	4	5	4	3	2	1	0
rTaq 1 <sup>u</sup> /ul	1	2	3	4	5	6	7
	100ul						

target DNA, 800nm primer, 10u Tag:

C	35ul	10x PCR buffer	✓
	10.5ul	50mM MgCl <sub>2</sub>	✓
	7ul	10mM 4 dNTPs	✓
	242.5ul	H <sub>2</sub> O	✓
	7ul	rTaq (5 <sup>u</sup> /ul)	✓

bx	12	13	14
er	4ul	4ul	4ul anchor
	anchor	407	4ul 407
0	94	4	0
	92	92	92

To Page No. \_\_\_\_\_

sed &amp; Understood by me,

Dolan

Date

6/30/95

Inv nted by

Recorded by

Cannon

Date

6-22-95

From Page No. \_\_\_\_\_

Δ [Target], use 400nM primers and 5u Tag/100ul  
for 7 rxns

[D]: 70ul 10x PCR buffer ✓  
21ul 50mM MgCl<sub>2</sub> ✓  
14ul 10mM 4 dNTPs ✓ \* dilute m13 RF to 0.5 pg/ul  
14ul 20uM anchovy ✓  
14ul 20uM 407 ✓  
462ul H<sub>2</sub>O ✓  
7ul rTag 5u/ul ✓  
20ul 1 pg/ul m13 RF  
20ul TC ✓

Tube #	15	16	17	18	19	20
m13 RF (0.5 pg/ul)	1	1.5	2	6	10	14 ✓
H <sub>2</sub> O	13	12.5	12	8	4	0 ✓
[D]	86					→ ✓

RI digestion of template:

3ul 1 pg/ul m13 RF ✓  
5ul 10x PCR buffer ✓  
3ul 50mM MgCl<sub>2</sub> ✓  
13ul H<sub>2</sub>O ✓  
1ul CcoRI 10u/ul ✓  
25ul

37°C, 30'

8.3ul 8.3ul 8.3ul  
91.7ul E →

3ul ✓  
5ul ✓  
3ul ✓  
13' ✓  
1ul H<sub>2</sub>O ✓

37°C 30'

same ✓

1 pm - 1.50 pm

21 22 23

24

25

26

To Page 1

With ss d & Und rst od by me,

Dat

6/30/95

Inv nt d by

R cord d by

Dat

6-29-95

DB Olamp

Protein Pamb

Diagram illustrating the structure of a 16-bit address (bits 15 down to 0):

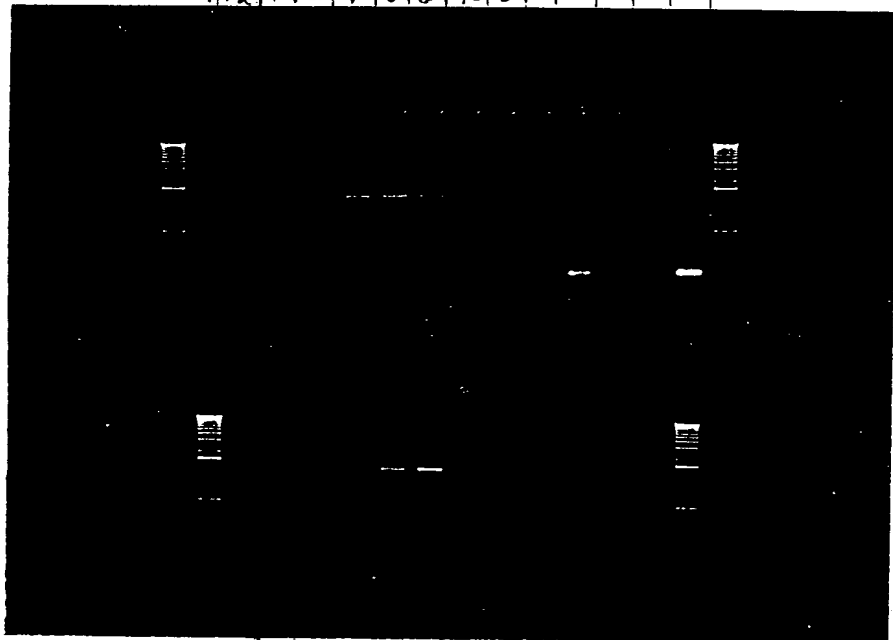
- Bits 15-12: 400 males
- Bits 11-8: 300 males
- Bits 7-4: 30000 primers
- Bits 3-0: 100 males
- Bits 15-12: units tag
- Bits 11-8: anchor
- Bits 7-4: 407
- Bits 3-0: anchor + 407

Address sequence (bits 15 down to 0):

1 2 3 4 5 6 7 8 9 10 11 12 13 14

11 12 13 14 15 16 17 18 19 20 21 22 23 24

anchor & 407 are primers



15	16	17	18	19	20	21	22	23	24	25	26
----	----	----	----	----	----	----	----	----	----	----	----

0.5 0.75 1 3 5 7 +CCORT -CCORT  
pg target

**To Page No.**

ad & Understood by me,

Date \_\_\_\_\_

**Invented by****Date**Recorded by                     

6-25-55

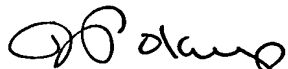
From Page No. \_\_\_\_\_

Results -

1. lag is inhibitory after 100  $\mu$ l PCR. Best results at 1 and 2 units
2. yields improve with increasing target
3. both primers present is required to make primer dimers
3. its not happening substrate or one primer self annealing to another copy of the same primer.

conclude the anchor primer should be OK for most PCR's

Witnessed &amp; Understood by me,



Date

6/30/95

Invented by

Recorded by

Date

6-2995

To Page 1

23mer. mp19 ssDNA

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

63

ge N (see P17, 9)

213 mp19 0.26  $\mu$ g/ $\mu$ l  
 .79 nmol nt/ $\mu$ l  
 .109 pmol circle/ $\mu$ l

200  $\mu$ l

226. (157 nmol nt total)

21.7 pmol circle  
 0.594 nmol nt/ $\mu$ l total  
 43.5 pmol primer total

23mer 5 ng/ $\mu$ l  
 2.66 pmol primer/ $\mu$ l

66.1  $\mu$ l

266  $\mu$ l

23mer  
 mp19  
 circles = 2

70°C, 5 min

cool at room temp 40 min

(for 60  $\mu$ l 23mer mp19)  
 conc in 100  $\mu$ l Rxn

mp19 is 10 mM Tris pH 7.4  
 5 mM NaCl  
 0.1 mM EDTA

6 mM Tris pH 7.4  
 3 mM NaCl  
 .06 mM EDTA

note Cheng 1X (P20, 10) =

20 mM Tris pH 9  
 7.5 mM KOAc  
 2% DMSO  
 1.05 mM MgOAc  
 8% glycerol

So buffer in 23mer mp19 will alter the reaction condition a little since 60  $\mu$ l 23mer mp19 is needed per 100  $\mu$ l Rxn

in future need more concentrated DNA

To Page No. \_\_\_\_\_

Read & Understood by me,

Date

Invented by

Date

30/6/95

6/30/95

Recorded by

6-29-95



Percent  
of zero time  
on P122/9

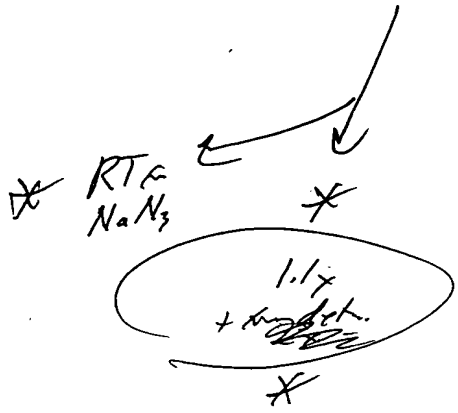
ag No.			u/a relative to 10g (#10-14)		
1	1597.00				
2	1752.00				
3	1760.00				
4	7709.00				
5	6150.00	} 6760 ave	.030	94%	
6	6422.00				
7	4510.00				
8	5085.00		.025	72%	
9	5662.00				
10	8620.00	} - 8931 CPM ave	↑ .04 (by definition) ↓		
11	9351.00				
12	8531.00				
13	8321.00				
14	9832.00				
15	5618.00	} 5632 ave	5632 / 8931 = .04	.025	-0.25 / .023 = 10.9%
16	5895.00				
17	5384.00				
18	5128.00	} 5215 ave	5215 / 8931 = .04	.023 u/a	.023 / .023 = 100%
19	5036.00				
20	5481.00				
21	3989.00	} 3673 ave	its been at -20°C for 1 month + 6 Free Thaws it came from tube #11 above (P122/9-6) conclude -20°C losses activity	P34.75 for threat	54% recover from .03 u/ml measured above (#4-6)
22	4058.00				
23	2971.00				
24	5931.00				
25	5591.00	} 5921 →	1 month -20°C from 5-8-95 1.1X (#15-17 above)	no activity lost for 1 month at -70°C	101%
26	6242.00				
27	5891.00				
28	5381.00	} 5712	from 1.1X of 5-8-95 (corrected 1.1X - 17 above)		
29	5865.00				
30	5644.00				
31	5407.00	} 5440	started with 1.1X (stored out at 40°C) of 5-8-95 (see #15-17 above)	conclude no loss of units for 50 Free Thaws	97%
32	5271.00				
33	5362.00	} 5405			96%
34	5494.00				
35	5361.00	} 5638			100%
36	5556.00				
37	6159.00				
38	5200.00				
39	138.00				
40	287.00				
41	137.00				
42	1014.00				
43	960.00				
44	1092.00				
45	395.00				
46	110131.00				
47	110429.00				

ave - BKGD = 627 CPM = 27.3 pmoL [32.8 u/ml] agrees with 36 u/a  
L-ig Flynn got out & got P.13. May 70. 7 u/a  
BKGD of 2.5 may be wrong. The is stable until more data is available  
2.1 mix  
2.1 mix  
[68.9 CPM/pmoL]

3 batch?

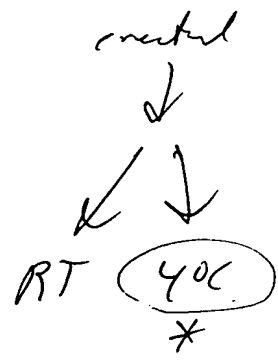
①  
17 sample  
organt biff. formulation -

created  
40C RT -200C 27/1



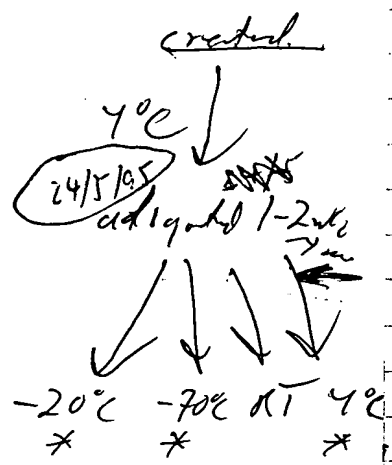
8x  
thund  
\*

②  
Date  
field test



May 8, 95 5:

③  
("new" on P34, 10)  
500ml batch  
lot



then  
make aliquots  
20ul each  
at -200C  
only 1 or 2 freeze then

The unit array \*

notes from  
Joe Jones

R Jones  
6-27-95

ag N — specific activity =  $(109,973 \text{ cpm}) \times \frac{100 \mu\text{l rxn}}{2 \mu\text{l spot}} = 275 \frac{\text{cpm}}{\text{pmol (nt)}}$   
 background = 188 cpm (5000 pmol) 4

Turnover (pmol)  
 $\left( \frac{\text{cpm} - \text{background cpm}}{\text{specific activity}} \right) \left( \frac{100}{2} \right) \left( \frac{20}{70} \right)$

CPM1

5' 774.00  
 10' 1379.00  
 15' 2170.00  
 5' 1031.00  
 10' 1588.00  
 15' 2241.00  
 5' 893.00  
 10' 1731.00  
 15' 1890.00  
 5' 788.00  
 10' 1365.00  
 15' 1836.00  
 5' 752.00  
 10' 1055.00  
 15' 1732.00  
 5' 636.00  
 10' 1140.00  
 15' 1448.00  
 5' 854.00  
 10' 1458.00  
 15' 2083.00  
 829.00  
 1512.00  
 2124.00  
 980.00  
 1612.00  
 2249.00  
 1182.00  
 2028.00  
 2271.00  
 1040.00  
 1816.00  
 2521.00  
 944.00  
 1729.00  
 2032.00  
 1087.00  
 1641.00  
 2701.00  
 917.00  
 2146.00  
 2530.00

$\frac{774-188}{275} \times 100 = 228 \quad 213$

433

721

307

509

747

256

561

619

218

428

599

205

315

561

163

346

458

242

462

689

233

481

704

288

518

749

361

669

757

310

592

848

274

560

671

327

528

914

265

712

851

$\bar{x}_{5'} = 229 \pm 45 \quad (20\%) \rightarrow$  Large error, next time  
 $10' = 436 \pm 86 \quad (20\%)$  cut out dATP + dADP  
 $15' = 627 \pm 101 \quad (16\%)$  spot in order to correct  
 for spotting error

$\bar{x}_{5'} = 293 \pm 45 \quad (15\%)$   
 $10' = 580 \pm 84 \quad (15\%)$   
 $15' = 785 \pm 88 \quad (11\%)$

195.00  
 162.00  
 169.00  
 194.00

no enzyme

186.00  
 189.00  
 223.00

background for turnover  
 $n = 7 \quad \bar{x} = 188.3 \pm 19.8 \quad (\sim 10\% \text{ error})$   
 $= 6.8 \text{ pmoles}$

To Page No. \_\_\_\_\_

Used & Understood by me,

*Dolan*

Date

6/30/95

Inv. nted by

Recorded by

*Penelope C. mls*

Date

6/30/95

Fr m Page No. <sup>adjust V<sub>f</sub></sup>  
back to 2661  
23. mp19 0.164 <sup>1.95</sup>  $\mu\text{g}/\mu\text{l}$   
0.594 nmol nt/ $\mu\text{l}$   
P. 63  
5x Chevy (P21, 10)  
4 dMPS 10 mM each  
2 <sup>32</sup>P dATP 10 mCi/ $\mu\text{l}$  (Amersham)  
H<sub>2</sub>O  
mix A  
264  $\mu\text{l}$  264 ✓  
88  $\mu\text{l}$  108 ✓  
2.2  $\mu\text{l}$  2.7 ✓  
1  $\mu\text{l}$  1.23  
78  $\mu\text{l}$  153.5 ✓  
431.2 529.2 (see p. 65)  
(5.4 P-xms)  
23.7  
29 nmol nt/  
\* 20.7  $\mu\text{g}/\mu\text{l}$   
65.6  
\* see P55 f  
change to on  
of DNA plasm  
(50  $\mu\text{M}$  each  
see P55 f  
change to on  
of DNA plasm  
(50  $\mu\text{M}$  each  
see P55 f  
change to on  
of DNA plasm

Reactions

	(1)	(2)	(3)	(4)	(5)
stop tube *	1-8	9-16	17-24	25-32	33-40
mix A	98 $\mu\text{l}$				2 $\mu\text{l}$ Epicenter storage buff
TFI/Vent = 0.18 units (opieuk TFI 5-17-95)	2				
Vent lot #17 = (opened 2-24-95)					
0.09 u/ $\mu\text{l}$ = 0.18 u		2			
0.5 = 1 u			2		
2 (no dilution) = 4 u				2	
V <sub>f</sub> = 100 $\mu\text{l}$					

note: can  
to Vent also  
with gaps  
on P 20  
} dilute  
opieuk  
TFI into  
buffer

68°C in 9600, remove 8  $\mu\text{l}$  to 8  $\mu\text{l}$  killing solution P.1  
spot 2  $\mu\text{l}$  on PET and 10  $\mu\text{l}$  on GFC  
\* note: 20  $\mu\text{g}/\mu\text{l}$  23. mp19 is ~16% as much total DNA as  
gapped DNA in Tag unit assay (500  $\mu\text{g}/\mu\text{l}$ ) however  
m13 is almost all ssDNA substrate while gapped DNA  
may have ~20 ssDNA gaps.

at 5 10 20 40, 60, 70, 100, 120 min  
\* spot DuPont on PET

From Page No. \_\_\_\_\_

Turnover  $(-284 \text{ pmol})$  percent turnover percent incorporation  
 (100)  $(\frac{\text{nmol incorporation}}{\text{nmol input DNA}})$

min

icenter	5	1	445.00	83	1.4	24	note: it app there is no T/O at c since T/O stops incorporation stops ~ 20 min
TFI	10	2	1588.00	672	7.8	53	
vent	20	3	3904.00	1266	13.6	50	
	40	4	6787.00	3352	22.4	49	
	60	5	6818.00	3368	21.3	52	
	80	6	7009.00	3468	22.4	54	
	100	7	7002.00	3462	22.4	50	
	120	8	7164.00	3546	21.4	55	
	5	9	333.00	25	—	0	note: turnover low and ends in for TFI/Vent. conclude most are not full. since low per incorp.
18 u	10	10	350.00	54	—	0	
Vent	20	11	381.00	50	—	0	
	40	12	521.00	122	37	0.9	
	60	13	832.00	282	43	1.6	
	80	14	1097.00	415	45	2.2	
	100	15	1474.00	615	45	2.7	
	120	16	1928.00	847	53	3.2	
	5	17	296.00	6	2	1.0	← about 25 max incorp 13.8% / 5- (55% seen at 120 min) 30 still max are not full
1 unit	10	18	508.00	115	16	2.6	
Vent	20	1	1322.00	535	21	6.8	
	40	2	3242.00	1524	52	13.8	
	60	3	6116.00	3006	42	17.6	
	80	4	8505.00	4238	45	22.1	
	100	5	11510.00	5788	52	22.9	
	120	6	13872.00	7004	52	27.3	
	5	7	750.00	—	—	—	more conclusions 1. T/O is occurring at an opt high rate (~20-22%) for TFI/ considering ~30:1 TFI/Vent (in units) 2. it appears that substrates act 3' end accumulate during time probably at hairpins (on MISS rather than at full length (since T/O stops shortly after incorporation stops even for 1
4 units	10	8	1728.00	—	—	—	
Vent	20	9	4617.00	—	—	—	
	40	10	9106.00	—	—	—	
	60	11	11531.00	—	—	—	
	80	12	12228.00	—	—	—	
	100	13	12432.00	—	—	—	
	120	14	11890.00	—	—	—	
	5	15	269.00	—	—	—	284 are AMP background
no	10	16	278.00	—	—	—	
enzyme	20	17	240.00	—	—	—	
	40	18	276.00	—	—	—	
	60	19	293.00	—	—	—	
	80	20	274.00	—	—	—	
	100	21	307.00	—	—	—	
	120	22	331.00	—	—	—	

3. Incorp plateau for Vent alone (~27% incorp) is lower than for TFI/Vent (~55%)

With ss d &amp; Und rst od by m

Date

7/7/95

Inv nt d by

R corded by  
sawyer Pamb

Dat

7-1-55

T Pag N

Polars

# Results of P64

Project No. C 23.7 n mol nt input ss M13 DNA  
 Book No. Percent substrate copied  
 67

Age No.	Incorporation	p mol	
23	632.00		
24	37364.00		
25	37879.00		
26	106114.00		
min	27	104061.00	
mply	28	34.00	
5	29	57217.00	57217
10	30	76607.00	76607
20	31	114434.00	11797
40	32	112213.00	11797
60	33	120505.00	11797
80	34	123172.00	12423
100	35	115839.00	12697
120	36	125999.00	11942
5	37	1441.00	12990
20	38	600.00	96
40	39	1390.00	(9)
60	40	2498.00	70
80	41	4176.00	207
100	42	5526.00	377
120	43	6777.00	517
5	44	7820.00	642
20	45	2449.00	753
40	46	6061.00	252
60	47	15599.00	624
80	48	31777.00	1602
100	49	40517.00	3276
120	50	50876.00	417
5	51	52552.00	5245
20	52	62653.00	5417
40	53	36563.00	6459
60	54 #38	260.00	
80	55	83243.00	7582
100	56	58704.00	6052
120	57	82715.00	7527
5	58	73558.00	7585
20	59	69056.00	7119
40	60	65008.00	6701
60	61	502.00	
80	62	591.00	
100	63	450.00	
120	64	960.00	
5	65	307.00	
20	66	563.00	
40	67	463.00	
60	68	80862.00	
80	69	75044.00	
100	70	81620.00	

ave BKGD = 5-12

ave 79175

194 CPM/pmol nt

To Page No. \_\_\_\_\_

s d & Understood by m , Polamp	Date 7/17/95	Inv nted by <i>[Signature]</i>	Date 7-1-95
		R c rded by <i>[Signature]</i>	

# BEST AVAILABLE COPY

Project N

Exhibit L-124

m13 PCR system: more optimization of  
[primer 407], annealing temp. and [target]

B k N

Appl. No. 09/558,421

69

Page N

x A, enough for 28 runs: 28.0ul 10x PCR buffer ✓  
8.4ul 50mM MgCl<sub>2</sub> ✓  
5.6ul 10mM dNTPs ✓  
2094.4 ul H<sub>2</sub>O ✓  
5.6ul T<sub>aq</sub> 5u/ul ✓  
2520ul

template dilution:  
to 50pg = 1ul stock m13 RF  
370ug/mL  
+ 99ul T.C.  
mix ✓  
3.1ul + 7.3ul T.C. ✓  
→ 7.4ul of 50pg/ul

13 RF 3ul of 10pg/ul 9ul 10pg/ul 6ul of 50pg/ul ✓  
12ul 18 24 12.18ul 2.4 12.18 2.4 ✓  
15ul 9ul 3ul 3ul 0 12ul, 6ul, 0ul ✓  
12.18 2.4 12.18 2.4

13.3 1-3 4-4 7-9  
3ul of 10pg/ul 9ul 10pg/ul 6ul of 50pg/ul ✓  
12ul 18 24 12.18ul 2.4 12.18 2.4 ✓  
15ul 9ul 3ul 3ul 0 12ul, 6ul, 0ul ✓

x A 270ul → 270ul → 270ul →  
300ul →  
divide into 3 tubes, 1 for each annealing temp.  
a, b, c

a = 53°	10pg m13 RF	4 a 53° 30pg m13	7 a 53° 100pg m13
b 55°	✓ 400nm primers	b 55° 400nm primers	b 55° 400nm p
c 57°		c 57°	c 57°
a = 53°	10pg m13 RF	5 a 53° 30pg m13	8 a 53° 100pg m13
b 55°	✓ 400nm primer	b 55° 400nm p	b 55° 600nm p
c 57°		c 57°	c 57°
a = 53°	12pg m13 RF	6 a 53° 30pg m13	9 a 53° 100pg m13
b 55°	✓ 800nm primers	b 55° 800nm p	b 55° 800nm primers
c 57°		c 57°	c 57°

To Page No.

sed & Understood by me,

Polamp

Date

7/7/9

Invented by

Recorded by

Date

7/5/95

70

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

1% agarose gels:

6g agarose  
600 mL 1x TAE

4.5ul 10mg/ml CtBr

M13RF: 10 pg

30 pg

100 pg

primer (nm) 400 400 800

cycles

30 25 20 15 10 5

30 25 20 15 10 5

30 25 20 15 10 5

30 25 20 15 10 5

30 25 20 15 10 5

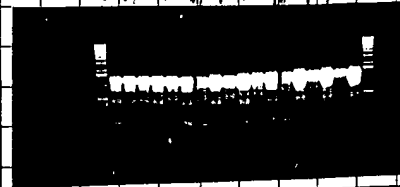
30 25 20 15 10 5

top

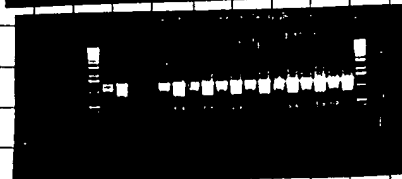
57°C C 1-9

55°C B 1-9

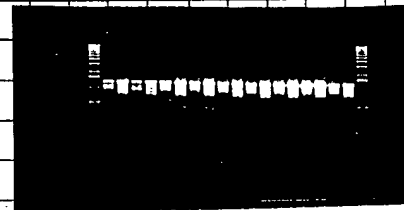
53°C A 1-9  
bottom



5



5



5

## Conclusion

1. no advantage to >400 nm primer
2. more target improves yield and specificity
3. 57°C is most specific

Carlynn Combs  
7/6/95

T Page 1

Witnessed & Understood by me,

*DPolam*

Date

7/7/95

Invent d by

*[Signature]*

Date

7/5/95

Recorded by

*[Signature]*



Page No. \_\_\_\_\_

pg target

10

30

100

primer (nm)

400	600	800	400	600	800	400	600	800
130 35	130 35	130 35	130 35	130 35	130 35	130 35	130 35	130 35

cycle #

anneal  
temp

57°C

55°C

55°C

To Page No. \_\_\_\_\_

is d &amp; Understood by me,

Polamp

Date

7/7/95

Invented by

Rec rd d by

Dat

7-6-95

From Page No. \_\_\_\_\_

title: m13 PCR system: 4 annealing temperatures and 4 primer sets.

53°C, 55°C, 57°C, 59°C

anchor + 6681  
7069  
407  
806

purpose: We have established optimal [template] & [primers] for anchor + 407. Now we'll optimize annealing temp and cycle ~~x~~ for the other primers 6681, 7069, 806. ~~we do it~~ assuming that these other primers will work well with the [template] & [primer] that worked well w/ 407. Later, we'll titrate [Taq] & [Tne] for these primers.

background: • 59°C has not been tried before w/ any primers

- in an earlier expt 57°C worked best for 407
- [template] = 100 pg / 100 µl rxn - found to be best for 407
- [primer] = 400 nM - found to be best for 407
- [Taq] = 1 U / 100 µl rxn, more was inhibitory for 407  
2 U / 100 µl rxn will be tried for 806 which makes the longest product

• expected product sizes:

anchor + 6681 → 380 bp

+ 7069 → 768 bp

+ <sup>407</sup>~~356~~ → 1356 bp

+ 806 → 1755 bp

Completed 7/6/95  
Pmb

materials: m13RF 50 pg/µl, diluted on 7/6/95 NBH p.

Witnessed & Und rsted by m ,

OBblays

Dat

7/7/95

Inv nt d by

R c rded by

Paul m Pmb

Date 7/7/95

7/6/95

T Pag

procedure:

make a master mix for 21, 100ul rxns - containing everything but the primers:

(A) { 210ul 10x PCR buffer  
1654.8ul H<sub>2</sub>O  
43ul 50mM MgCl<sub>2</sub> Cf = 1.5mM  
42ul 10mM dNTP's Cf = 200uM  
42ul m13mp19RF 50pg/ul stock  
42ul anchor primer, 20uM Cf = 100pg/100ul rxn  
stock Cf = 400nM  
4.2ul Taq 5u/ul Cf = 1u/100ul rxn  
2058ul

remove 441ul and add 0.9ul Taq (5u/ul) - for 806 primer  
w/ 2u Taq/100ul rxn

is (1.5mL)	1	2	3	4	5
mer, 20uM stock	8ul 6681	8ul 7069	8ul 407	8ul 806	8ul 806
)	392ul				(B) →
	400ul				

divide into 4, 100ul aliquots in 9600 PCR tubes  
and put each tube in different 9600's set to different  
annealing temperatures 53° - Lab 15

55°C - Lab 16

57°C - SGT

*Paula* 7/6/95  
*Combs*

59°C - Lab 14 \* note that 30 cycle  
aliquots were taken

during ramp to 94°C

Pause the 9600's during later part of the 70°C, 2'  
extension to withdraw 25ul samples at 25, 30, 35 cycles -  
+ 2.8ul Blue Juice

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*Polamp*

Date

7/7/95

Invented by

Recorded by

*Paula Combs*

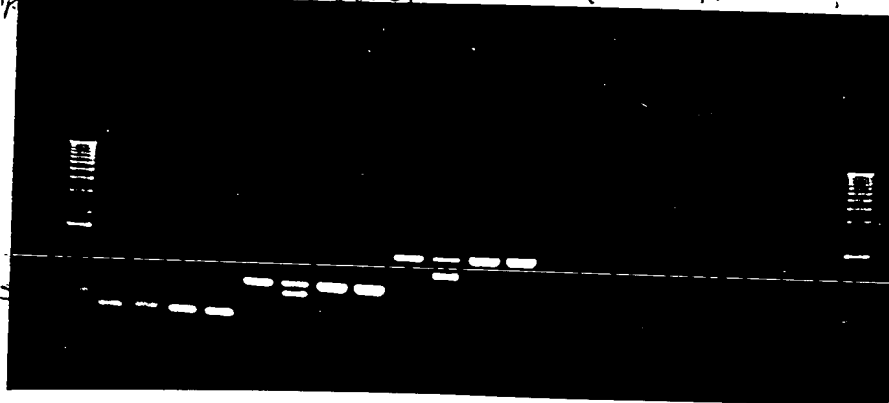
Date

7/6/95

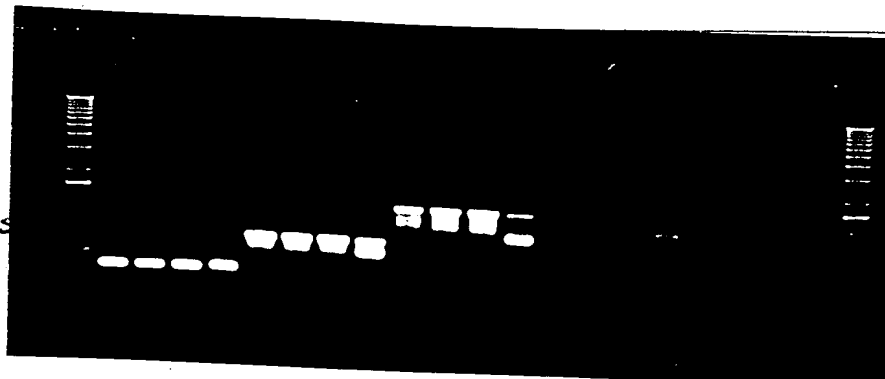
primer  
annealing temp  
6681 7069 407 1st run 806 2nd run 806

100

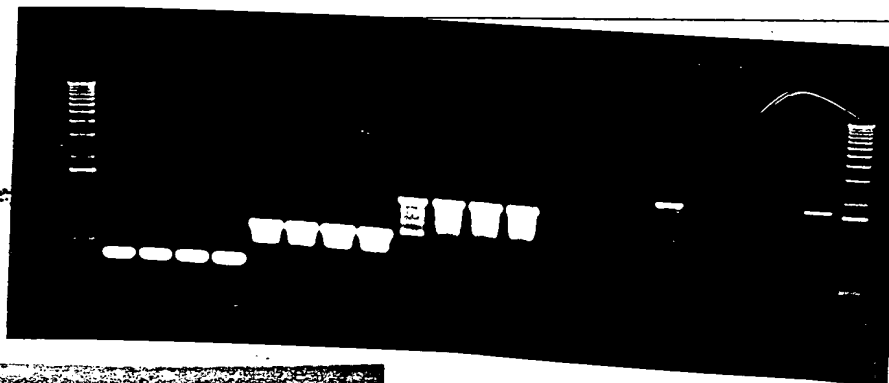
25 cycles



30 cycles

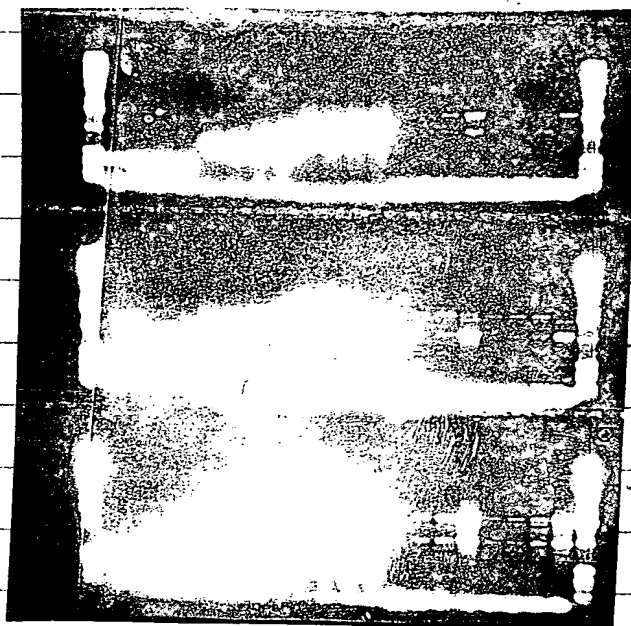


35 cycles



Note: 59°C  
cycles - I  
9600 during  
to 94°C so  
product is,  
ssDNA - acc  
for the 2,  
in 7069 & 407

Pauline  
Combs  
7/6/95



25 cycles

30 cycles

35 cycles

- 59°C annealing temp for 25 cycles gave a good product yield and the least nonspecific products for primers 6681, 7069, 407
- Try higher annealing temps for primer 806 because product is beginning to come up at 59°C

With ss d & Und rsto d by m ,

Pauline

Date 7/7/95

Inv nt d by Pauline Combs  
R corded by Pauline Combs

Date 7/7/95  
7/6/95

purpose: i) To optimize the [Tag]  $\rightarrow$  1  $\mu$ mol rxn was previously found to be inhibitory, so lower conc. will be tried - 0.25  $\mu$ M.

2) To see if the cell synthesizes any of the products expected w/ 6681, 7009, 7191, 407 primers + anchor primer - no product was made (p.44) when 5uTne/100uL was tried. The [template] and [primers] that were optimal for Tag will be used in the Tne PCR rxns. 0.1-1 u will be tried

- 25, 30, 35 cycle samples will be taken
- 59°C annealing temperature, 400nM primers, 100pg/100ul rxn mix

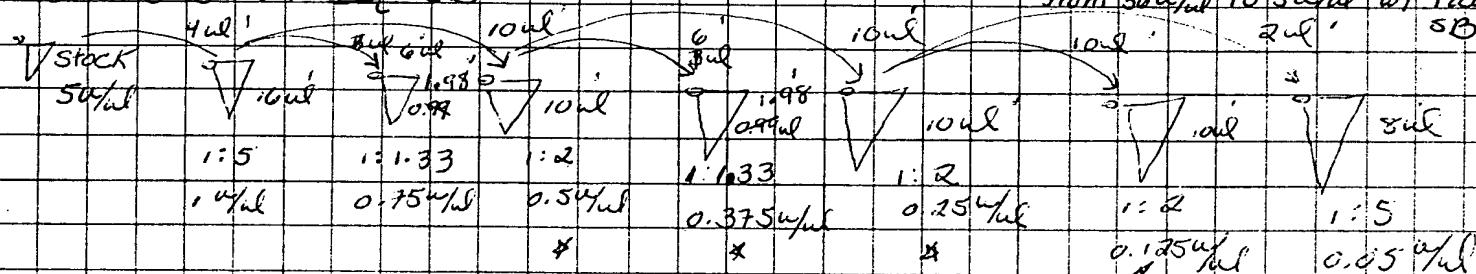
arterials:

mix	A	w/ 6681, for 11.5 rxns	885.5 $\mu$ l $H_2O$	
			115 $\mu$ l 10x PCR buffer	
			34.5 $\mu$ l 50mM $MgCl_2$	Cf = 1.5mM
			23 $\mu$ l 10mM dNTPs	Cf = 200 $\mu$ M
			23 $\mu$ l 20 $\mu$ M anchor	Cf = 400 $\mu$ M each
			23 $\mu$ l 20 $\mu$ M 6681 primer	
			23 $\mu$ l M13RF 50 pg/ $\mu$ l	Cf = 100 pg/rxn
			1127 $\mu$ l	see p. 69 for dilution of stock to 50 pg/ $\mu$ l lot FAS301

mix **B** same as **A** but use 23ul, 20um T069 primer ✓

$m \times [C]$  same as  $[A]$  but use 23uf, 20um 407 primer ✓

The dilutions in Tag SB: RT, rinse pipet, vortex 2sec — 5/7/95 Lys Flynn diluted from 344.1 to 500 w/ Tag



Also do same dilutions w/ Taq in Taq  $\frac{50}{50}$  - only  $\frac{50}{50}$  dil will be used

Step soln = 100ml EDTA → 80ul 0.5M EDTA - needed to Kill exo  
8x Blue Juice + 320ul 10x Blue Juice ice won't Kill it  
400ul

**To Page No.**

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Date \_\_\_\_\_

Invented by

Date \_\_\_\_\_

Po lamp

7/7/95

Recorded by

7/6/95

Fr m Page No. \_\_\_\_\_

run #	1	2	3	4	5	6	7	8 → 14	15-21
	98ul [A]						1	98ul [B]	98ul [C]
1.4% Tne	2ul							same as 1-7	same as 1-7
0.75% Tne		2ul							
0.5% Tne			2ul						
0.375% Tne				2ul					
0.25% Tne					2ul				
0.125% Tne						2ul			
0.05% Tne							2ul		
	100ul								

\* remove 25ul rxn after 25, 30, 35 cycles to 2.8ul Blue Juice

note: 59°C annealing temp, 10:40 AM - 2  
 { 94°C 15" } Lab 15  
 { 59°C 30" } program 74  
 { 70°C 2' extension } 9600  
 4°C final

run #	22	23	24	25	26-29	30-33
	98ul [A]				98ul [B]	98ul [C]
0.5% Tag	2ul				2	2
0.375% Tag		2ul			2	2
0.125% Tag			2ul		2	2
0.05% Tag				2ul	2	2

30 cycles only

\*\* remove 25ul to 2.8ul Blue Juice

\* remove 25ul of Tne rxns after 25, 30, 35 cycles during last part of 2min, 70°C elongation into 4ul stop soln in microtiter plate. The final [CDTA] = 10mM  
 [Blue Juice] = 1.1X

\*\* remove 25ul of Tag rxns after 30 cycles + 4ul stop soln

T Pag N

With ssed &amp; Understood by m ,

M Polamp

Date

7/7/95

Inv nt d by

R c rd d by

Radm. Pombi

Date

7/7/95  
7/6/95

7/7/95

Polany

10 R

$5 \times \text{Cheng (no LTP)} (P2, 10)$

100 ✓

100

10 mM DNTPr

10	✓
----	---

10	
----	--

(200,000)

Human spleen genomic  
DNA (HS #2 19/4)  
BO neg / sub

12.5 ✓

(100 ng / 5)

GAPDH(+) 2112, 10  $\mu$ m

20 ✓

600 nm

GARDH(-) 2113 10 $\mu$ m

20	✓
----	---

Mg O AC 100mm

2.5	✓
-----	---

$$\left\{ \begin{array}{l} Cf = 1. \\ \text{für } |B| \end{array} \right.$$

PCR DNA  
Xmn I 25-70 / 1

10	✓
----	---

 $2836,10 \mu m'$ 

20		

2837, 10  $\mu$  M

20		

$$H_2O$$
$$\begin{array}{r} 317.5 \\ \hline 470 \end{array}$$
$$\begin{array}{r} 317.5 \\ \hline 480 \end{array}$$

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
48									→								

48  $\longrightarrow$

The 5-7-95 (Liz)

0.5		2				
1.5			2			
2				2		
					2	2.5

2				
	2			
		2		
			2	2.5

The  $534/\mu\text{l}$  Adam 3-2-55  
ditto  $24/\mu\text{l}$

1	2
---	---

1	2
---	---

Tag 2 u/wl
------------

1	2
---	---

T	Pag	N
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10
11	11	11
12	12	12
13	13	13
14	14	14
15	15	15
16	16	16
17	17	17
18	18	18
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21	21	21
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89	89	89
90	90	90
91	91	91
92	92	92
93	93	93
94	94	94
95	95	95
96	96	96
97	97	97
98	98	98
99	99	99
100	100	100

Witnessed & Understood by me,

**Dat**  
7/14/95

Inv nt d by

Recorded by

**Dat**

7-10-95

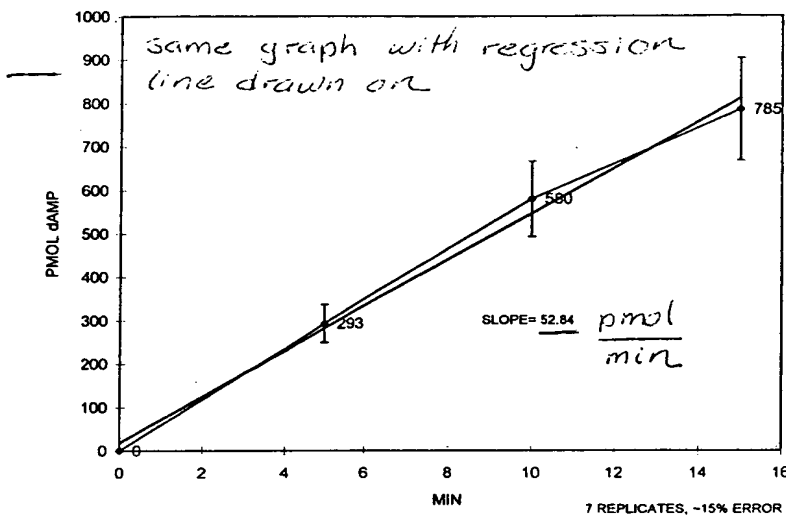
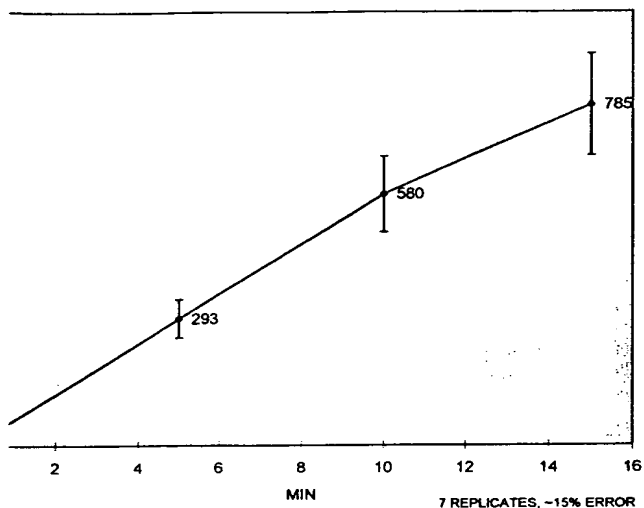


Sheet3 Chart 1

Sheet3 Chart 1

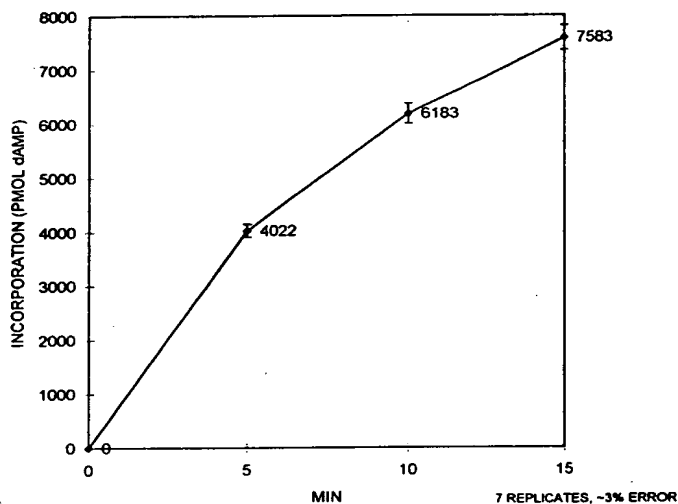
LT'S TivVent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)

LT'S TivVent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)



Sheet4 Chart 1

LT'S TivVent: POLYMERIZATION AT ZERO TIME POINT IN STABILITY STUDY (6/28/95)



calculations:

To Page No. \_\_\_\_\_

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Date

Invented by

Date

Recorded by

7/11/95

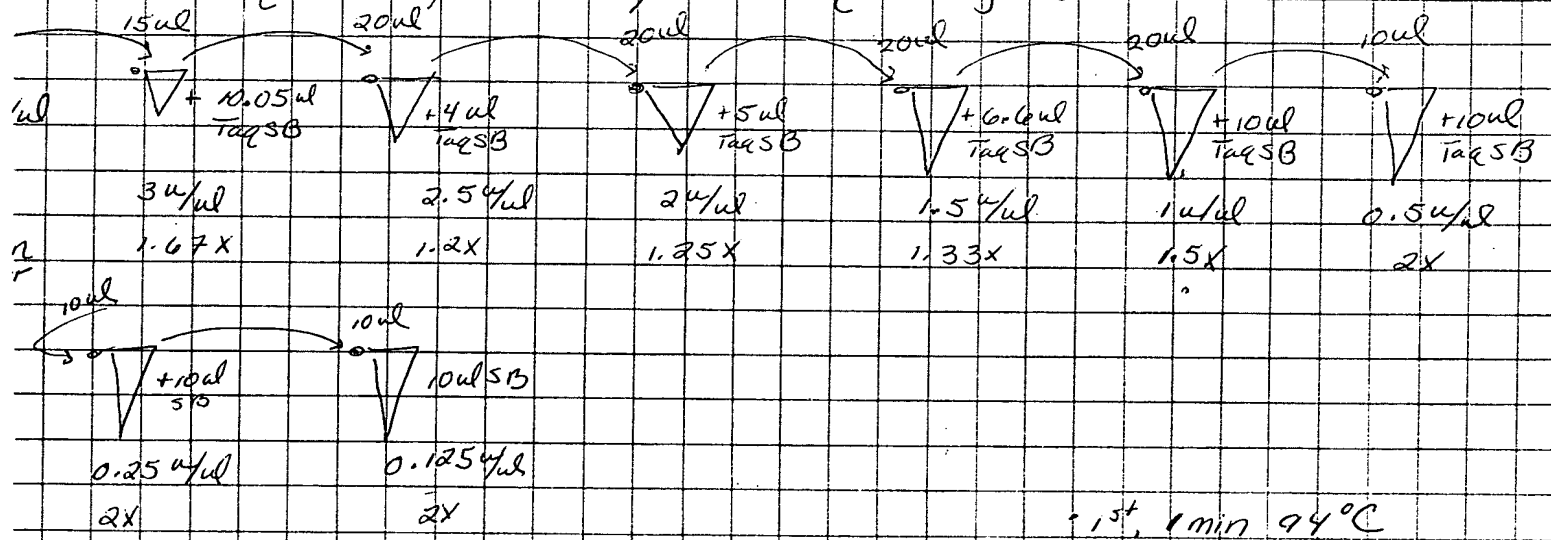
*Carolyn Combs*

age No. — purpose: Can we make the 380bp ml3 PCR product w/ Tne? Earlier p. 77 w Tne failed to make this product in 10x PCR buffer.

A for ~~17~~ rxns, 100ul per rxn: 340 300ul 5x Cheng buffer  
 34' 30ul 10mM dNTPs  $C_f = 200\mu M$   
 34' 30ul 50 pg/ul ml3mp19 RT in TC  $C_f = 100pg$   
 34' 30ul 20uM anchor primer  $C_f = 400nM$   
 34' 30ul 20uM 6681 primer  
 1190' ul H<sub>2</sub>O

1066 ul + 470 ul

tions of Tag + Tne (5/7/95 Lit) in Tag storage buffer (SB)



5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
93ul A									2ul Tne (5-7 95 Lit)							
2ul Tag	2								2							
		2								2						
			2								2					
				2								2				
					2								2			
						2								2		
							2								2	
								2								2
100ul																

• 15', 1 min 94°C  
 • 94°C 30" denat.  
 • 55°C annealing temp 30"  
 • 72°C exten 2'  
 • remove 25ul aliquots after 25, 30, 35 cycles  
 program 76  
 16 1pm - 4pm  
 23  
 75  
 \* remove 25ul p. 79  
 + 3ul STOP soln (w EDTA)  
 \* run 25ul

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE m13 PCR: 3 small products w/ Tne

84

From Page No. \_\_\_\_\_

purpose: To determine if Tne can make the 3 smallest m13 PCR products 380 bp, 768, 1356 bp, 380t using the conditions which worked for gapDH & PUC p80. Cheng buffer, 400uM p, 35 cycles, 55°C annealing temp & 94°C 30" denat, 100 p9/10, 72°C 2' extension

If the products are made, we can use these conditions for m13 primer extension experiments

materials: mix for 15, 50ul rxns

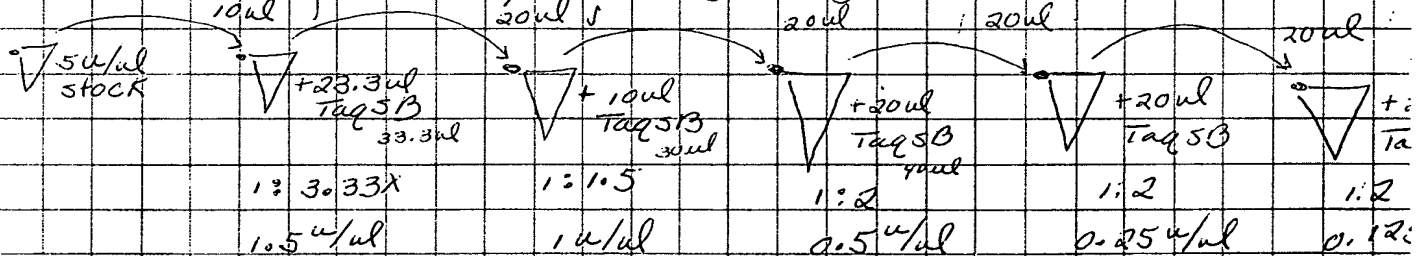
[A] 150ul 5x Cheng  
510ul H<sub>2</sub>O  
15ul 10mM dNTPs  
15ul 50p9/10 m13mp19 RF  
15ul 20uM anchor primer  
705ul

[B] = 235ul A  
+ 15ul 20uM 6681

[C] = 235ul A  
+ 15ul 20uM 7069

[D] = 235ul A  
+ 15ul 20uM 407

dilutions of Tne (5/7/95 Lig) in Taq storage buffer:



rxn 1 2 3 4 5 6 7 8 9 10 11 12 13

[B] 48 48 48

[C] 48

[D] 48

1.5uM Tne 2 2 2 2 2 2 2 2 2 2 2 2 2

1uM 2 2 2 2 2 2 2 2 2 2 2 2 2

0.5uM 2 2 2 2 2 2 2 2 2 2 2 2 2

0.25uM 2 2 2 2 2 2 2 2 2 2 2 2 2

0.125uM 2 2 2 2 2 2 2 2 2 2 2 2 2

start rxns on ice w/ en

Result on p. 86  
Only the 380t product was made.

Witnessed & Underst d by m ,

*[Signature]*

Date

7/14/95

Inv nt d by

*[Signature]*

R c rd d by

*[Signature]*

Date

7/12/95

To Page N

<sup>32</sup>P-23. M13 for TFI/vent

ig No. \_\_\_\_\_ same experiment as P. 64 except <sup>32</sup>P 23 instead of <sup>32</sup>PDA  
2 increasing T/O with time (P. 64-68) due to strong pause site  
visible on an agarose gel?

5 ng /  $\lambda$  23mer  
5x Kinase buffer  
PNK

35  $\mu$ l  
10.2  $\mu$ l  
1.2  $\mu$ l  
5

1.75 ng  
25 pmol 23mer

$\gamma$ ATP 10 mCi/ml 7-14-95

51.4  $\mu$ l

37°C, 30 min  $\rightarrow$  55°C, 5 min

51.4  $\mu$ l  
42.6  
10.6

H<sub>2</sub>O

M13 mp19 0.26  $\mu$ g/ $\lambda$   
0.79 nmol nt/ $\lambda$   
0.109 pmol circle/ $\lambda$

11.5 pmol  
circle tot

200

$\frac{23mer}{circle} = 2$

70°C, 5' cool slow

Cf = 0.41 nmol nt M13/ $\mu$ l

it will be ~23.7 total nmol nt / 100  $\mu$ l Rxn  
same as for P. 64

To Page No. \_\_\_\_\_

sed & Und rstood by me,  
Polansky

Date  
7/14/95

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R corded by

Date  
7-13-95  
RZ

From Page No. \_\_\_\_\_

(A)

3.5 R<sub>x</sub>ao32P23<sub>mem</sub> - mPIA (P87)

171.1

0.41 nmol nt /  $\mu$ l0.566 pmol acid /  $\lambda$ 

5X Chumy (no dNTPs)

70

P21, 10

10 mM dNTP<sub>2</sub>

1.75

C<sub>f</sub> = 5  $\mu$ mH<sub>2</sub>O

100.15

V<sub>f</sub> = 343(use 98  $\mu$ l / 100  $\mu$ l R<sub>2</sub>)

①

②

③

(A)

9  $\mu$ l

—————→

Tf1/Vent  
(equivalent Tf1)  
5-16-95

2

(1.88  $\mu$ l total in  
← its only 0.94  
equivalent units) due  
with Vent by NirTf1 lot 31010A-502  
1  $\mu$ l (equivalent units)1.88  $\mu$ l(1.88 unit H<sub>2</sub>O)Vent 2  $\mu$ l  
lot #17  
opened 2-24-95

2

(4 units) at 1.2 is  
more Vent than in Tf1,  
in order to get full length  
products)68°C. Remove 8  $\mu$ l at 1, 2, 5, 10, 15, 20, 40, 60, 9  
to 1  $\mu$ l 10X 'blue juice' mM E

Run on agarose (same as P56, 9)

T Page N

Witnessed &amp; Understood by m ,

D. Polansky

Dat

7/14/95

Invent d by

R c rded by

R. J. Park

Dat

7-14-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Exhibit L-132

Appl. No. 09/558,421

From Page No. \_\_\_\_\_

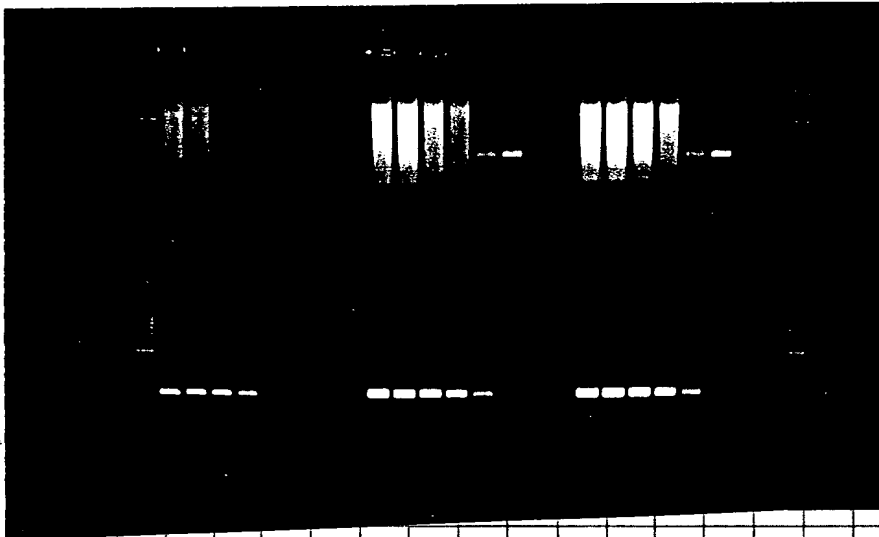
1% agarose gel

top ~~empty~~

9-16	9-16	9-16
25	30	35

 inc 9-16 decreasing eny

1-8	1-8	1-8
25	30	35

 cycles Tag 1-8 decreasing eny - run into gel 1st  
 The from p. 80


same photo as p.  
 taken from a further  
 distance to capture  
 the ~~with~~ bottom of  
 gel

To Page N .

Witnessed &amp; Understood by me,

Dat

7/14/95

Investigated by

Recorded by

Pamela P. Smith

Dat

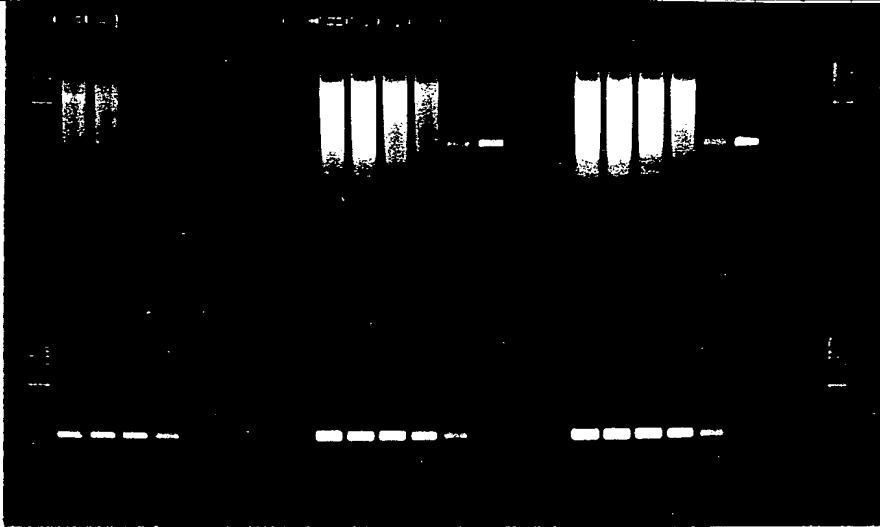
7/14/95

Page No. \_\_\_\_\_

Cheng buffer, 380bp product expected

units      25 cycles      30 cycles      35 cycles      1 pool rxns

6 5 4 3 2 1 .5 .25    6 5 4 3 2 1 .5 .25    6 5 4 3 2 1 .5 .25



Tne

rTaq

- 1 unit Tne is optimal, 35 cycles 21.5
- 6 units rTaq gave the most product for all cycle #3

To Page No. \_\_\_\_\_

ss d &amp; Understood by m ,

r Polamp

Date

7/14/95

Inv nt d by

Rec rd d by

Cauler Comb

Date

7/14/95

ml3 PCR: A PCR buffer into Cheng  
buffer

ag N \_\_\_\_\_

2.1: 3g agarose.

4 mL 50X TAE.

294 mL H<sub>2</sub>O

300 mL wt 514.5g w/ magnet

+ 20 mL CHBr<sub>3</sub>

purpose: To find out what component(s) of Cheng  
are important for making the broad  
smear (vs narrow, low MW smear made  
w/ PCR buffer)

reagents: [A] for 35 rxns = 35  $\mu$ L 20 mM anchor primer  
35  $\mu$ L 20 mM 6681 primer  
35  $\mu$ L 10 mM dNTPs  
910  $\mu$ L H<sub>2</sub>O  
1015

[B] w/ target

507.5  $\mu$ L of [A]

+ 17.5  $\mu$ L 500g/mL  
ml3 RF

[C] no target DNA

507.5  $\mu$ L of [A]

+ 17.5  $\mu$ L H<sub>2</sub>O

\*Nens 210/95

[D] = mix of 200 mM Tricine + 10.05 mM MgOAc to add KOAc to = 10X working stock  
200  $\mu$ L of 1M Tricine pH 9 (from Nens)  
+ 10.05  $\mu$ L of 1M MgOAc  
+ 789.95  $\mu$ L H<sub>2</sub>O  
1 mL

[E] = mix of 166.6 mM Tricine pH 9, 708 mM KOAc & 75 mM MgOAc  
is 8.33X stock 166.6  $\mu$ L 1M Tricine pH 9  
354  $\mu$ L 2M KOAc  
8.75  $\mu$ L 1M MgOAc  
470.65  $\mu$ L H<sub>2</sub>O

Tne (5-7-95) Liz 5  $\mu$ L diluted to 0.2  $\mu$ L w/ Taq storage buffer + 148.8  $\mu$ L Tne

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	→ 28	12/7/95
buffer	5																
NgCl <sub>2</sub>	1.5																
		5	5	5													
1 KCl		10															
1 KOAc			2.35	5.88													
						6	6	6	6	6	6	6	6	6			
lycerol						2	5	8						8	8	8	
MSO	8.5	0	7.65	4.12						5	.75	1		5	.75	1	
	8.5	8.5	7.65	4.12	9	7	4	8.5	8.5	8.25	8.25	8	0.5	.25	0		
Tne	5																
	50 $\mu$ L																

To Page No. \_\_\_\_\_

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Polaris

Date

7/14/95

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Date

7/14/95



From Page No. \_\_\_\_\_

Buffer components

Tris pH 8.4 20

KCl 50

MgCl<sub>2</sub> 1.5

Tricine pH 9 20

MgOAc 1.05

KOAc 20 50 85

glycerol 20 5 8

DMSO 1 1.5 2 1 1.5 2

Lane #

1

2

3

4

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352

Project N \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

is  $^{32}$ P primer incorporated into either  
the "primer dimer" or smear acc.  
The on P. 86 ?

From Page No. _____	(- Target)	(+ Target)	( $^{32}$ P M. KOAC)	
	(A)	(B)	(C)	(10 Rens /
1 M Tris pH 8.4	10			20 mM
3 M KCl	8.33	8.33	-	(50 mM)
50 mM MgCl <sub>2</sub>	15			(1.5 mM M)
1 M Tris pH 9		10	10	20 mM
MgOAC 50 mM		10.5	10.5	(1.05 mM)
M13 RF 50 pg/ul	-	10	10	(50 pg / 500 PCR
10 mM dNTPs	10	10	10	21.35 <u>2 M KOAC</u>
H <sub>2</sub> O	366.7	361.2	<del>350</del> 348.25	
	VR = 410	410	-410	

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

(A)

41  $\mu$ l

(B)

41  $\mu$ l

(C)

41

6681 10 $\mu$ M	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
6681 10 $\mu$ M			2			2			2			2			2	
301 10 $\mu$ M	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
6301 10 $\mu$ M		2			2			2			2			2		2

Cf = 400 nM

The 5-75 (Liz) 5  $\mu$ l0.2  $\mu$ l  $\mu$ l diluted

in Tag SB

35 cycles as per P 86

note for 3  $\mu$   
80% PAGE ita  
0.08  $\mu$ l  $^{32}$ P primer

mix 20  $\mu$ l PCR with 2  $\mu$ l BJT, 100 mM EDTA load 10  $\mu$ l on 80% AG  
as per P 56 9 150 V start ~ 2 PM (?)  
Movers as per AK book 7 start 4:30

To remain 30  $\mu$ l PCR add 15  $\mu$ l cycle mix stop, load 5  $\mu$ l  
on 80% PAGE (wells 1-17 are PCR reactions)

for sequencing Rxn use  $^{32}$ P 6681 - mp19 ss DNA (P 71) as per  
(P 27, 4)

T Page 1

Witness d &amp; Und rsto d by m ,

S. Polans

Date

7/14/95

Inv nt d by

R c rded by

Dat

7-14-95

<sup>32</sup>P 6681 and <sup>32</sup>P 6301  
anchon

Project N \_\_\_\_\_  
Book No. \_\_\_\_\_

g N	(can see P150, 9)	①	②		
100 $\mu$ M 6301 (anchon)	10.51			✓	Cf = 10 $\mu$ M Cf
100 $\mu$ M 6681		1.51		✓	
<sup>32</sup> P $\gamma$ ATP 10 mCi/ $\mu$ l	10	10		✓	
2/7-14-95					
1 $\mu$ l PPK 10 $\mu$ l	1	1		✓	
5X Kinase buffer	3.1	3.1		✓	
	15.66 $\mu$ l	15.6			
0 $\mu$ M 6301 cold	78.3				
$\mu$ M 6681 cold	93.98	78.3			

Cf at 1X Kinase buffer  
35 mM Tris pH 7.6  
50 mM KCl  
5 mM MgCl<sub>2</sub>

ie dilute hot primer with 5 parts cold primer  
so contribution to PCR of P90 is:  
primer in PCR

MgCl <sub>2</sub>	0.83	0.033
Tris pH 7.6	5.8 mM	0.23 mM
KCl	8.3	0.33

<sup>32</sup>P 6681 = mp19 for sequence

mp19 0.26  $\mu$ g/ $\mu$ l 0.109 pmol code/1 10  $\mu$ l (1.09 pmol code)

<sup>32</sup>P 6681 10  $\mu$ M primer 1 10 pmol primer  
70°C cool slow

for 3  $\mu$ l on PAGE is ~0.04  $\mu$ l primer

To Page No. \_\_\_\_\_

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Polamp

Date

7/14/95

Invented by

Recorded by

Date

7-14-95

From Page

1.5 mM

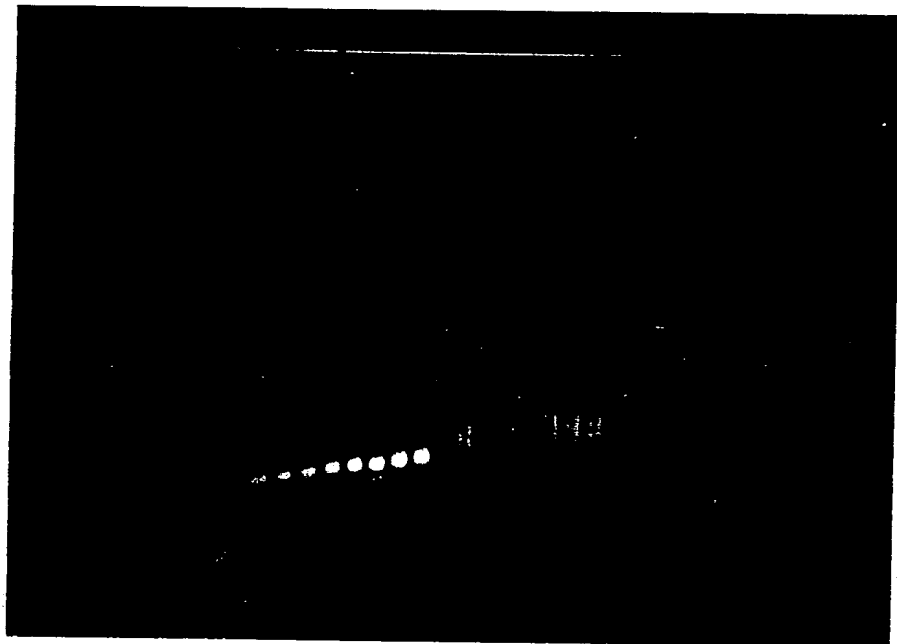
MgCl<sub>2</sub>

1.05

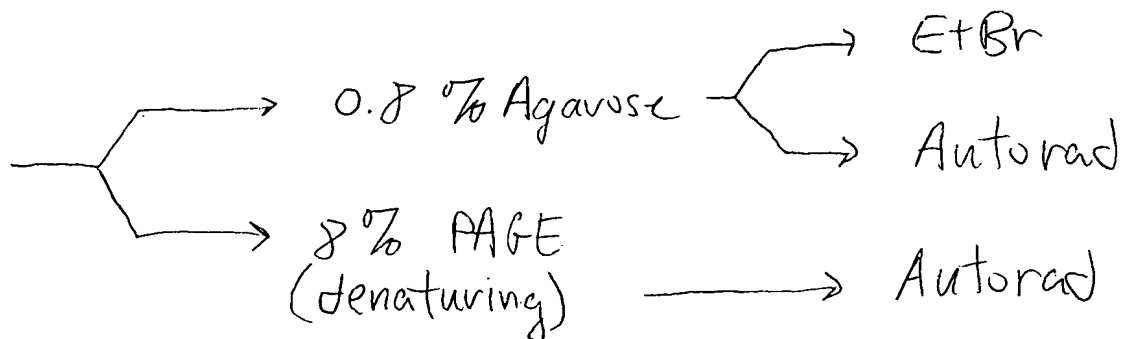
MgOAc

Tris  
pH 8.4Tricine  
pH 9

KOAc 85 mM



PCR  
Tne  
32P primers



With ss d &amp; Und rst d by m ,

Date

Inv nted by

Dat

R c rd d by

7-14-97

**PAGE 93 OF NOTEBOOK WAS BLANK**

1.5 mm  $MgCl_2$

The "smear" condition was 20mM Tricine pH 9

1.05 mm MgOAc

fr 15<sup>8.4</sup> vs  
? } [mg]  
mg OAC. 6

2) Is the "smear" or "primer dimer" due to DNA contamination of the prep? <sup>mg DNA</sup> rxns w/o D.

180  $\mu$ l 0.2  $\mu$ g/l Tne (5-7-95 Lig)  $\rightarrow$  7.2  $\mu$ l Tne stock 5  $\mu$ g/l  
+ 172.8  $\mu$ l Tag storage buffer

100mM Tris 8.5 \* note this is a change from the original 8.4 "dimer" condition 100mM Tris 8.5 (P1)

100  $\mu$ l 1M Tris 8.5 (RL)  
900  $\mu$ l  $H_2O$

100mM Tricine pH 9	100ul 1M Tricine pH 9 (Nern 2/10/95)
	900ul H <sub>2</sub> O

$$\frac{5.25}{10.5} \text{ mm } \text{MgCl}_2$$

210ul	50mm MgCl <sub>2</sub>	- 105ul
790ul	H <sub>2</sub> O	- 895ul

7.5  
15 mm  $MgCl_2$

300ul 50mM  $MgCl_2$  - 150ul  
700ul  $H_2O$  - 850ul

5.25  
~~10.5~~ mm MgOAc

10.5ul	1M MgOAc	5.25	10.5ul	5.25
989.5	ul H <sub>2</sub> O		1989.5ul	994.7
			2ml	

7.5  
15 mm MgOAc

15ul	1m MgOAC	-	15ul	7.5ul
485ul	H <sub>2</sub> O	-	1985ul	992.5ul
			<u>2mL</u>	

T	Pag	N
1	1	1
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99	99	99
100	100	100

**Witnessed & Undertaken by me,**

**Dat**

**Invent d by**

## Dat

**R c rd đ b y**

7/14/95

g N .

res:

A = 30ul 10mM dNTPs ✓  
 29.88ul 3M KCl ✓  
 726.12ul H<sub>2</sub>O (1st) ✓  
 792ul

[B] template + primers

374ul [A] ✓  
 17ul 20uM anchor primer ✓  
 17ul 20uM 6681 primer ✓  
 17ul 50 pg/ul M13RF ✓

425ul

use 25ul per 50ul rxn → This will give

t up each rxn in duplicate

er components:

		small smear condition = acp buffer	tricine instead of Tris	1.05 Mg <sup>2+</sup> instead of 1.5	MgOAc instead of MgCl <sub>2</sub>	change [Mg <sup>2+</sup> ] standard	broad smear condition	Tricine w/ high [Mg <sup>2+</sup> ]	Tricine w/ MgCl <sub>2</sub>
rxn #		1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16
Tris pH 8.4		✓		✓	✓	✓			
KCl		✓	✓	✓	✓	✓		✓	✓
n MgCl <sub>2</sub>		✓	✓						
Tricine pH 9			✓				✓	✓	✓
um MgCl <sub>2</sub>				✓					✓
n MgOAc					✓			✓	
nm MgOAc						✓	✓		

400nM primers  
 50 pg template  
 50mM KCl  
 200 uM dNTPs  
 in 50ul rxns

To Page No. \_\_\_\_\_

ed &amp; Understood by me,

D. Lamp

Date

7/24/95

Invented by

R. Corded by  
C. Lamp

Dat

7/14/95

**From Page N .\_\_**

[illegible]

**With ss d & Und rstood by me,**

**Dat**

Inv nt d by

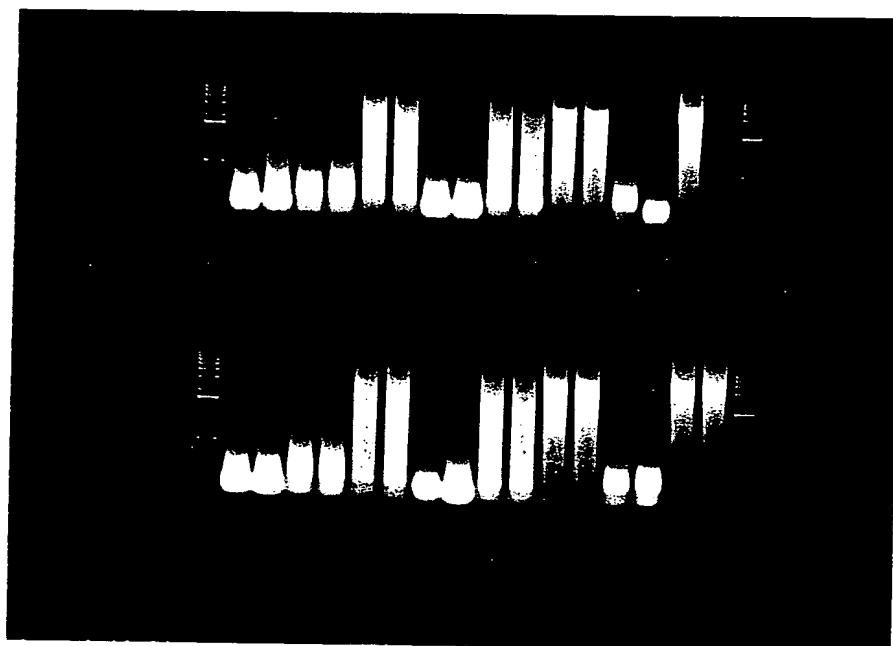
## Dat

T Pag



ag N. \_\_\_\_\_

> Tris w/ 1.5 mM  $MgCl_2$   
 > Tricine w/ 1.5 mM  $MgCl_2$   
 > Tris w/ 1.05 mM  $MgCl_2$   
 > Tris w/ 1.05 mM  $MgOAc$   
 > Tris w/ 1.05 mM  $MgOAc$   
 > Tricine w/ 1.05 mM  $MgOAc$   
 > Tricine w/ 1.5 mM  $MgOAc$   
 > Tricine w/ 1.05 mM  $MgCl_2$



template +  
primers

no DNA  
ie no template  
no primers

ecomb

5/14/95 CC

all at 50 mM KCl

lower concentration of  $Mg^{2+}$  in Cheng vs PCR buffer is responsible for the broader smear. The Tris/tricine pH difference and  $MgCl_2$  vs  $MgOAc$  do not affect the transition from small<sup>narrow</sup> to broad smear.

its model: distance between primers  
 low  $[Mg]$   
 high  $[KCl]$   
 low  $[Tne]$

short = small narrow smear  
 long = broad smear

✓ by decreasing primer annealing  
 ✓ by inhibiting Tne from binding primers

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

Date

Invented by

Date

Recorded by

20/10/95

7/21/95

Environ Ecomb

7/14/95

N. \_\_\_\_\_

15 9600 - 94°C 1min  
 94°C 30 sec  
 55°C 30 sec } 35 cycles  
 72°C 2min  
 4°C hold

p rxns by adding 7.5 ul of Stop soln = 8x Blue Juice Cf = 1x  
 100mM CDTA Cf = 12.5mM CDTA

u. Stop soln made for future use = 50% glycerol  
 100mM CDTA (10x)  
 0.6x TAE  
 BøB

for 5mL: 2.5mL 100% glycerol  
 1mL 0.5M CDTA  
 1.5mL 1x TAE = 30uL 50x TAE stock + 1470uL H<sub>2</sub>O  
 + pinch of BøB

To Page No. \_\_\_\_\_

Read &amp; Understood by me,

Polay

Date

7/14/95

Invented by

Recorded by

C. L. L. L.

Date

7/15/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

Buffers

Exhibit L-134

Appl. No. 09/558,421

From Page No. \_\_\_\_\_

Buffer C, 1 L ref NB 9, pg 162

✓ 17.1 mL 1M K phos monobasic  
 ✓ 7.9 mL 1M K phos dibasic  
 ✓ 80 mL glycerol  
 ✓ 149.12 g KCl CF = 2 mL  
 ✓ 0.2 mL 0.5M EDTA  
 ✓ 350 µL 14.3M BMC - add  
 ✓ qs to 1 L w/ H<sub>2</sub>O

1M K phos monobasic  
 68.045 g  
 qs to 500 mL  
 1M K phos dibasic  
 114.115 g  
 qs to 500 mL

+ 1 mL of 50% Tween 20 + NP40 - post filter det 1 mL + BMC

Buffer D, 8 L ref NB 9 p 182

✓ 200 mL 1M Tris 7.5  
 ✓ 1.6 mL 0.5M EDTA  
 ✓ 640 mL glycerol  
 ✓ 2.8 mL 14.3M BMC - add  
 ✓ 29.8 g KCl CF = 50 mL  
 qs to 8 L

make 0.01% NP40 + Tween 20 for dialysis  
 + 0.05% for Heparin column - filter 1 L + add 1 mL 5% NP40 + Tween 20 + 350 µL

Buffer C, 500 mL ref NB 9 p 182

✓ 12.5 mL 1M Tris 7.5  
 ✓ 0.1 mL 0.5M EDTA  
 ✓ 40 mL glycerol add  
 ✓ 0.175 mL β-me  
 ✓ 174.5 g KCl CF = 2 mL  
 ✓ qs to 500 mL

det 0.05% final  
 1 mL/L

+ 5 mL 50% Tween 20 + NP40 after filtering

T Pag N

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Oolamp

Dat

7/18/15

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Davidson Romo

Dat

7/18/15

small scale ext  
and  $\text{AmSO}_4$  (can see P163, 9)

Project N \_\_\_\_\_

Book No. \_\_\_\_\_

101

ig N \_\_\_\_\_ 7/18/95

20ml Tag extract buffer (P.167, 3) with 0.05% each NP40 / Tween 20  
250ul 10mM PMSF

17.6ul 14.3M  $\beta$ ME

25ul Tween 20  $\rightarrow$  50% mix of both  $\rightarrow$  5ml each

25ul NP40

4g cells -70°C Thorton shelf, Lab 16 - chip off 9503-15-764-D1-001K

20ml Tag extract buffer mix w/ spat in 50ml Tne 5 Pol 85g

20ml 0.2g cells/ml + 10ml pipet Falcon

cate tune - on XL2020  $\rightarrow$  0 turn, turn small to min  
to tune } then min 1-5 minimize, not over 70  
(stop)

9x 30sec pulses in ice- $\text{H}_2\text{O}$  bath, ~1min between  
pulses - should turn browner

75°C in Falcon -  $\text{H}_2\text{O}$  bath, then cool in ice- $\text{H}_2\text{O}$

Ke  $\frac{\text{CF}}{200\text{mM}}$  NaCl + 5% PEI to CF = 0.5%  $\rightarrow$  16mL vol extract in grad  
58.44g/m cylinder

$$.2 \frac{\text{mole}}{\text{L}} \times .016 \text{ L} = 0.0032 \text{ M}$$

30mL centrifuge tube

$$\frac{\times g}{58.44 \text{ g/m}} = 0.0032 \text{ M}$$

0.187g NaCl ✓

1.5% 16mL  $\frac{1.5\text{mL}}{5\%}$  of 5% PEI while extract is  
stirring in c. tube, 1 drop/sec

r 15min, 4°C

in 15min, 15K 5534, 4°C (~2700xg) DNA, cell debris & heat-denatured  
proteins will ppt

cant into 25mL grad cylinder;

up = FI' fraction

nd  $\text{AmSO}_4$  to help it go into soln

$$13.25 \text{ mL of FI}' - .2 \text{ mL} = 13.05 \text{ mL}$$

$$\Rightarrow 2.297 \text{ g ammon.}$$

To Page No. \_\_\_\_\_

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From Page No. _____		remove 200ul aliquots of each fraction - eppendorf on ice add AmSO <sub>4</sub> , spin 15 min, spin down 15K. 15; remove aliquot remeasure volume		
S30	AmSO <sub>4</sub>	vol (mL)	g AmSO <sub>4</sub> slowly, stir 15'	
Fr I'	0	13.05	2.297g	
S1	30%	13.8	0.414g	
S2	35	13.5	0.182g wrong 0.223g more added	
S3	40-40	13.5 13.75	0.405g 0.223g 0.436g aliquot had x	
S4	45	13.5	0.41g	
S5	50	13.5	0.432	
S6	55	13.4	0.442	
S7	60	13.2		
S8	65			
S9	70			

mix: 150ul 0.5M Tapes 9.3 x 2 = 300ul 0.5M Tapes 9.3

A } 6ul 1M MgCl<sub>2</sub>  
 { 50ul 3M KCl

12ul 1M MgCl<sub>2</sub>  
 100ul 3M KCl

made 2

3mL

204ul mix A

60.3ul <sup>32</sup>P dCTP 10mCi/mL

60ul 10mM dNTPs

405ul 3.7mg/mL gap activated DNA

2.523mL H<sub>2</sub>O

3.2mL for 46.7 runs with, use 48ul/rxn

6.4mL

412ul mix A

12.6ul <sup>32</sup>P dCTP 10mCi/mL

120ul 10mM dNTPs

810ul 3.7mg activated DNA

5.046mL H<sub>2</sub>O

6.4mL

1:100 in Tag dilution buffer

52ul dil + 48ul reaction mix

10', 74°C

stop w/ 10ul 0.5M EDTA

spot 20ul on GEC filters

2ul aliquot + 198ul Tag dil bu

50ul rxn

+ 10ul stop

spot 20ul

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Page No. \_\_\_\_\_

$$\left( \frac{\text{cpm}}{\text{specific activity}} \right) \left( \frac{\text{cpm}}{\text{cpm}} \right) = \text{p mole}$$

 VM CPM  
 19195 c. comb

		pmol	units/ul
1 no enj	71.00	7.55	
2 FI	4679.00	498	7.47
3 SI	5411.00	576	
4 S2	5860.00	623	
5 S3	5434.00	578	
6 S4	3558.00	377	
7 S5	1394.00	148	
8 S6	299.00	31.8	→ 55% ammonium sulfate
9 mix	45046.00	2ul	$\bar{x} = 45048$
0 mix	44957.00		
1 mix	45141.00		

$$\text{specific activity} = \left( \frac{45048 \text{ cpm}}{2 \text{ ul}} \right) \left( \frac{50 \text{ ul rxn}}{2 \text{ ul}} \right) = \frac{28.2 \text{ cpm}}{\text{p mole nt}}$$

$$\frac{20,000 \text{ } \cancel{5000} (4) \text{ p mole nt}}{10,000 \text{ p mole dCTP}}$$

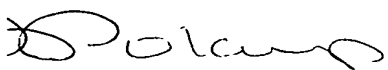
$$\frac{498 \text{ p mole}}{10 \text{ n mole}} \times 3 \stackrel{\text{for } 10'}{=} \frac{\text{units (200)}}{2} = 7.47 \frac{\text{units}}{\text{ul}} \text{ in FI'}$$

$$100,000 \text{ units} / 3.5 \text{ g cells} = 28,000 \text{ u/g}$$

Liq - 22,000 u/g

To Page No. \_\_\_\_\_

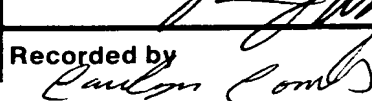
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Date

7/24/85

Invented by



Recorded by

Paulson Comb

Date

7.11.85

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Large scale (81.5 g cells)

The prep (can see P176, 9)

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

105

ig N — except use detergent in Tag ext buffer

81.5 g

9503 -15-714 D1-001R The 5 vol

326 ml

Tag ext buffer (P167), 3: with  
(Brown Temp) 0.05% each  
Tween 20 / NP40

(Cf  $\approx$  0.2 g / ml cells  
stir cells in RT buffer in beaker  
strain thru cheesecloth

Mininganlin

10,000 PSI

1 pass

1/2 mL = FRI The, spun down cells 15 min 4°C - sup = FRI The

75°C 15 min (total time after Temp reaches 75°C)  
cool fast in ice ~~shaking~~ shaking

vol = 405 ml

NaCl added to Cf 200 mM

= 4.73 g ✓

$$\frac{200 \text{ mmole}}{\text{L}} \times 0.405 \text{ L} = 81 \text{ mmole} \\ = 0.081 \text{ mole} \\ \times \frac{58.44 \text{ g}}{\text{mole}} = 4.73 \text{ g}$$

PEI for Cf = 0.5% add

45 ml 5% PEI pH 7.4 ✓ add

add dropwise, stir 15' more ✓

spin GSA 18,000 RPM 30 min ✓

ammonium & divide in 2

2 bottles

Sup = FRI' / PET

To Page N \_\_\_\_\_

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Date

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7-18-95



**From Page N .\_\_**

vol of FRI'/PEI sup = 355 mL  
removed 1/2 mL = FRI'/PEI

$$\frac{351g}{1000mL} = \frac{124.6g}{355mL} + \text{ref R. Scopes. Protein Purification p. 304}$$

add 124.605 g ammonium sulfate (ground up) for 55% sat  
added slowly over ~ 15 min while stirring at 4°

stirred 15 min,  $4^{\circ}\text{C}$

spin down pellet 13K, 30 min GSA RC-5B, 4°C → pellet contains  
(spin in two bottles to produce 2 pellets of equal size) Inc DNA polym.  
saved sup at 4°C in case activity didn't come down  
also 1/2 ml aliquot

respun 5K 5min to pull as much liquid off the pellets as possible  
put the 2 pellets at  $-70^{\circ}\text{C}$

FPLC method for S200 column  
method 5 bank 2

```

METHOD 5      BANK 2
0.00 CONC /B 0
0.00 CONC /B 0
0.00 BLK MIN 1.
0.00 PORE /SFT 6
0.00 PORE /SET 6
0.00 VAL E. POS 1
0.00 VAL E. POS 2
0.00 CONC /B 0
0.00 BLK MIN 1

```

0	conc % B	0.0
0	conc % B	0.0
0	mL/min	1.5
0	port. set	6.1
0	port. set	6.1
0	valve pos	1.1
0	valve pos	2.1
400	conc % B	0.0
400	mL/min	1.5

**To Page**

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Equilibrate 10ml supernatant  
and S200

Project N \_\_\_\_\_  
Book N \_\_\_\_\_

S200  
column

107

ge N - wash with H<sub>2</sub>O, (HiTrap Pharmacia)  
equilibrate a 10ml blue column (used once and stored in  
20% EtOH P1919)  
at 3 col vol/hr = 0.5 ml/min  
for 2 1/2 hr (= 7 col vol) with buffer B

wash S200 with H<sub>2</sub>O (180 ml vol P178,9)  
Equilibrate with buffer B  
at 2 col vol/hr  
= 90 ml/hr  
= 1.5 ml/min } for S200 col with equilibrate  
at 0.6 ml/min  
= 500 ml in 14 hr (= 3 col vol)

S200 column

Resuspend one of the two AmSO<sub>4</sub> pellets of P.106  
(i.e. 0.5 of the total material from 81.5g cells on P105)  
in buffer B (~~and~~ containing detergent) (as per P178, 9)

final vol = 0.89 ml spun out insoluble material  
in microfuge 15'  
remove 20 ul to assay later AmSO<sub>4</sub> resuspend

Load by gravity on 180 ml S200.

elute at 1/2 col vol/hr (i.e. 1.5 ml/min) in buffer B

spin = 2A, 2 min/min, 1.5 ml/min, 3 ml/frn 2 min/frn

To Page No. \_\_\_\_\_

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Date

7/24/91

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7-20-91

From Page No. \_\_\_\_\_

unit assay on fractions 3-17 from S200 column

2ul fraction + 0.25ul Tag dil buffer  $\Rightarrow$  1:31.622ul + 0.25ul Tag dil buffer  $\Rightarrow$  1:31.62 total of 1:1000 dil

2ul of 1/1000 dil + 48ul mix A p. 102

+ a no eng control

10min 74°C - temp varied between 72-77°C

10ul STOP 0.5M CDTA

20ul on GPC - wash 1x 10%, 3x 5% TCA, 2x EtOH

dry 20min, count

specific activity =  $\frac{\text{cpm}}{\text{S.A.}} \left( \frac{60\text{ul}}{20\text{ul}} \right) = 28.2 \text{ cpm/pmol n}$ 

SAM	CPM1	pmol		unit
1 no eng	41.00	4.36	546 were cloudy	no eng.
fraction 23	52.00	5.53		3
from 34	51.00	5.43		4
S200 45	45.00	4.79		5
56	153.00	16.3		6
67	721.00	76.7		7 34/5
78	634.00	67.4		8 30
89	2027.00	216	pool fr 9, 10 = 8 ml total	9 97 1/2
910	4597.00	489	$\hookrightarrow$ = 317,250 units loaded	10 220
1011	301.00	32	onto Blue Sepharose	11 4
1112	344.00	36.6		12
1213	820.00	87.2		13
1314	208.00	22.1		14
1415	531.00	56.4		15
1516	321.00	34.1		16
1617	254.00	27.0		17

unit def:  $\frac{\text{unit}}{\text{ul}} \rightarrow \text{inc. } 10,000 \text{ pmole nt in } 30 \text{ min} \Rightarrow \frac{2027}{2\text{ul}} \cdot \frac{216 \text{ pmol}}{\text{ul}} \cdot 1000 \times \frac{1}{10,000} = 1.08$

$1.08 \times 10^5 \frac{\text{pmol}}{\text{ul}} \left( \frac{30 \text{ min}}{10 \text{ min}} \right) = 3.24 \times 10^5 \frac{\text{pmole}}{\text{ul}}$

$= 32.4 \frac{\text{ul}}{\text{ul}} \times 3000 = 97,200 \text{ units}$

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D. Polansky

Date

7/20/95

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R. Polansky

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7-20-95

gg N —  
Blue Sepharose

load fr 9, 10 177 S200 (P10P)  $V_f = 6 \text{ ml}$   
 $1 \text{ col vol/hr} = 10 \text{ ml/hr} = 0.17 \text{ ml/min}$

Wash O/N

7 col vol buffer B total = 70 ml

( $0.08 \text{ ml/min} \times 70 \text{ ml in } 15 \text{ hr}$ )

collect  $10 \times 7 \text{ ml fractions of wash}$   
 $7 \text{ min/fr}$

THOD 5 BANK 2

0.00	CONC %B	0.0
0.00	CONC %B	0.0
0.00	ML/MIN	0.08
0.00	PORT. SET	6.1
0.00	PORT. SET	6.1
0.00	VALUE. POS	1.1
0.00	VALUE. POS	2.1
0.00	CONC %B	0.0
0.00	ML/MIN	0.08

FAIL. M=5 B=2  
 11.31 RE= 11.31

C. Combs  
 7/21/95

gradient: will scale down gradient  
 for TPI of P182, 9 &

for 10 ml Blue col

200 ml total gradient (20 col vol)  
 of 50 mM - 1 M KCl

$3 \text{ col vol/hr} = 0.5 \text{ ml/min}$

$3 \text{ ml/fr}$

$6 \text{ min/fr}$

$1 \text{ mm/min}$

start ~ 7:45 AM

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7-21-95

39 N  
Blue Sepharose

load fr 9, 10 of S200 (P10P)  $V_f = 6 \text{ ml}$   
 $1 \text{ col vol/hr} = 10 \text{ ml/hr} = 0.17 \text{ ml/min}$

Wash O/W

7 col vol buffer B total = 70 ml

( $0.08 \text{ ml/min} \times 70 \text{ ml in } 15 \text{ hr}$ )

collect 10 x 7 ml fractions of wash  
 7 min/fr

THOD 5 BANK 2

0.00	CONC %B	0.0
0.00	CONC %B	0.0
0.00	ML/MIN	0.08
0.00	PORT.SET	6.1
0.00	PORT.SET	6.1
0.00	VALUE.POS	1.1
0.00	VALUE.POS	2.1
0.00	CONC %B	0.0
0.00	ML/MIN	0.08

FAIL. M=5 B=2  
 11.31 RE= 11.31

C. Combs  
 7/21/95

gradient: will scale down gradient  
 for TPI of P182, 9 &

for 10 ml Blue col

200 ml total gradient (20 col vol)  
 of 50 mM - 1 M KCl

3 col vol/hr =  $0.5 \text{ ml/min}$

3 ml/fr

6 min/fr

1 ~~mm~~ mm/min

start ~ 7:45 AM

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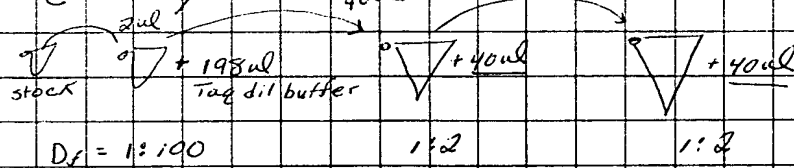
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7-20-95  
 7-21-95

From Page No. — 7/21 Unit assay

 $^{32}P$   $\alpha$  dCTP ref 7/14/95

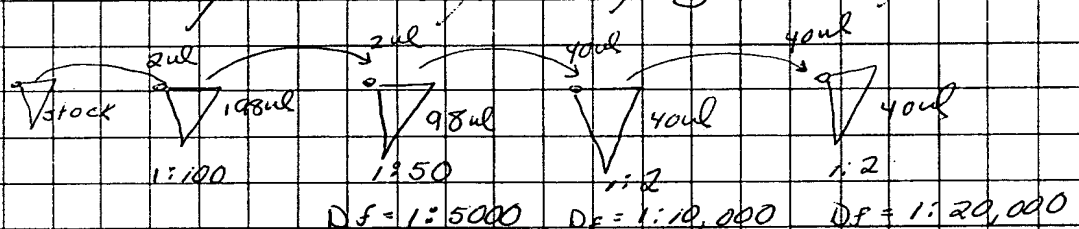
dilutions in Tag dilution buffer

• Fr T' / PET  
355 mL

①

②

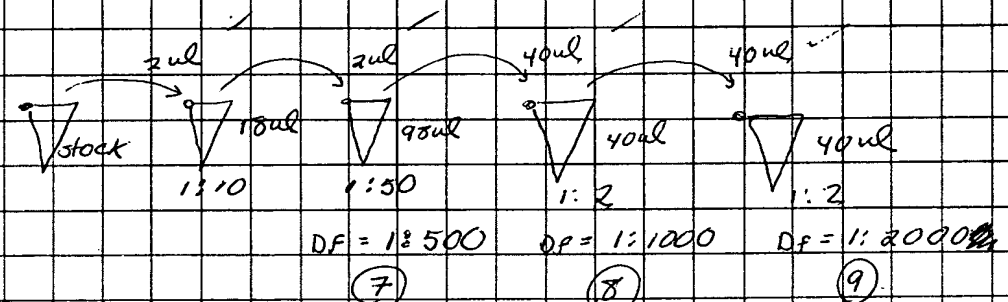
③

• Am SO<sub>4</sub> pellet  
0.89 mL  
resuspension $D_f = 1:5000$  $D_f = 1:10,000$  $D_f = 1:20,000$ 

④

⑤

⑥

• S200 pooled peak  
fractions 6 mL $D_f = 1:500$  $D_f = 1:1000$  $D_f = 1:2000$ 

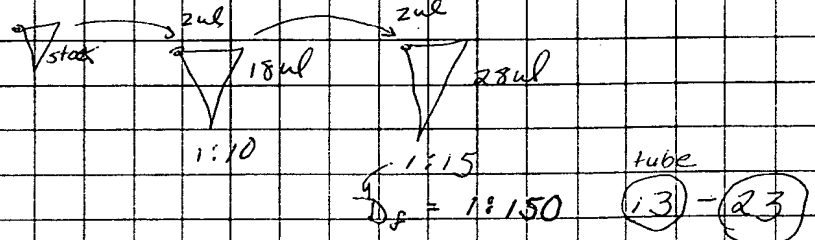
⑦

⑧

⑨

• Fractions from Blue  
Sephacose columnsundiluted wash fractions W/5 ⑩  
W/10 ⑪  
F/5 ⑫

peak elution fractions 10-20

 $1:10$  $1:15$  $D_f = 1:150$ 

tube

⑬ - ⑲

Start runs by adding 2ul of each dilution to 48ul A mix p. 102  
 74°C 10min  
 + 10ul 0.5M EDTA, wash & spot 20ul

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7/21/95

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Pamela Pomb

7/21/95

ig N .—

CFM1.

$$\frac{\text{pmole}}{\text{cpm}} = \text{specific activity}$$

1/100	4504.00
1/200	3334.00
1/400	1539.00
1/600	11945.00
1/800	8088.00
1/1000	4497.00
1/1500	9437.00
1/2000	5329.00
1/3000	3011.00
wash 5	108.00
wash 16	98.00
fr 5	107.00
10	651.00
11	813.00
12	1642.00
13	3668.00
14	7866.00
15	10929.00
16	6668.00
17	6788.00
18	5668.00
19	3724.00
20	2935.00

1. frn 13-17  
re 2nd (of 3) ml from each fraction 2.  $V_f = 10 \text{ ml}$

## Analysis of blue print

Analysis against 1800 ml buffer D (pH 8.0)

continued on p. 114

**To Page No.\_\_\_\_\_**

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7/21/97

**Invented by**

**Recorded by**

Recorded by *Carolyn Cant*

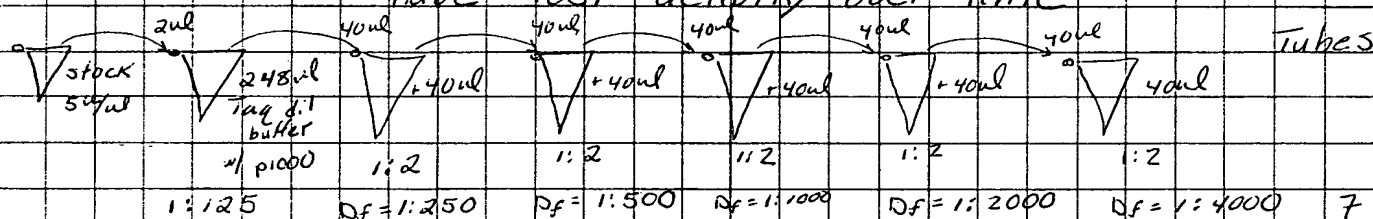
**Date**

7-2157

From Page No. — Unit assay on old Tne prep (5-7-95) side-by-side the new Tne pool which was eluted from Blue agarose column on 7/21/95 p.111 NB11. Sepharose

enzyme dilutions in Tag dilution buffer:

The (5-7-95 Lig) — ~~label~~ previously determined to be 5 u/l, but it have lost activity over time



same dilutions also made w/ Tne from Blue Sepharose column pool

start rxns w/ 2 u/l of each dilution into 48 u/l mix A <sup>ref</sup> p.102  
10' 74°C, stop w/ 10 u/l 0.5M EDTA, spot 20 u/l  
<sup>32</sup>P x dCTP ref 7/14/95

SAM	CPM1
1 1/125	2730.00
2 1/250	1638.00
3 1/500	790.00
4 1/1000	388.00
5 1/2000	232.00
6 1/4000	143.00
7 1/125	9450.00
8 1/250	5021.00
9 1/500	2872.00
10 1/1000	1669.00
11 1/2000	899.00
12 1/4000	503.00
13 no eny	67.00
C. Comb 7/22/95	

pmol 4 u/l  
352 543 544 6.45 } even ~ 7.0 u/l  
202 7.60  
93 7.50

207 31.0 }  
107 32 } 32.3 ave  
56 33.8

(= 193600 units total in blue pool fr 13-17)

23.2  
27.2 cpm/pmol  
as of 7-22-95



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From Page No. \_\_\_\_\_

check conductivity after dialysis of P111

10  $\mu$ l / ml H<sub>2</sub>Obuffer D 88  $\mu$ SHep col effluent 84  $\mu$ SDialysate 94  $\mu$ S

so conductivity is good and is similar to P183, 9 for TFA

1. Load on ~ 22 ml Hep equilibrated on with buffer D (P100)

at 0.67 ml/min (= 40 ml/hr = 2 col vol/hr)

2. wash 1 col volGradient (start ~ 9:30 AM)50 mM - 1.05 M KCl (0.67 ml/min)  
using buffer D and E (2 M KCl)

so 0 - 50% E

600 ml total gradient vol (~ 30 col vol)

so 50 - 700 mM KCl is in 20 col vol same as P185, 9

0.5 ml/min span = 2A

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Storage buffer

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

115

ag N \_\_\_\_\_

2 L buffer F (as per 91342. PRP)

2 M Tris pH 8	40 ml	✓
0.5 M EDTA	0.4 ml	✓
0.1 M DTT	2 ml	✓
0.03 NP40 Pierce	✓	✓
0.1% Tween 20 Pierce	✓	✓
Glycerol	1 L	✓
H <sub>2</sub> O		

2 L

Dialyze in 1 L for 5 hr. Change to another L for 1 hr.

Unit assay on fractions eluted from Heparin column:  
do 1 dilution (1:150) of fractions # 35-53 and a series of  
dilutions on the fraction with the maximum UV absorbance - #44

13 dilutions in Tag dilution buffer

Stock  $\xrightarrow{2 \mu\text{L}}$  298  $\mu\text{L}$  Tag dilution buffer p1000

1:150 for fractions 35-53, called H35-H53

and ~~1:125~~, 1:250, 1:500, 1:1000, 1:2000, 1:4000, 1:8000 of H44 - see dilutions on p 112

do make 1:125  
but don't do  
run w/ it

To Page No. \_\_\_\_\_

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7-27-95

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Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

pmol u/ml

fr 44 {

1:250	2180.00
2:500	938.00
3:1000	560.00
4:2000	398.00
5:4000	252.00
6:8000	146.00

274  
114  
55  
44

10.3  
8.5  
5.8 } ave 9.53 u/ml

specific activity  
= 19.1 cpm/p

Hopfr  
13150  
dilution  
35-53

no Enz  
fr 35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53

7	57.00
8	1024.00
9	699.00
10	1128.00
11	1127.00
12	2104.00
13	2484.00
14	2621.00
15	4760.00
16	3657.00
17	3507.00
18	4717.00
19	4120.00
20	3517.00
21	3280.00
22	2282.00
23	1820.00
24	1301.00
25	750.00
26	513.00

pmole

412  
748  
574  
550  
740  
447  
552  
515

$$\frac{412 \text{ pmole}}{2 \text{ ml}} \times 150 \times \frac{30}{10} = 9.27 \text{ u/ml}$$

$$\bar{x} = 13.3 \text{ u/ml} \pm 2.6 \text{ u/ml}$$

pool 41-47

(Pfrn) (1.37 ml/frn)

= 10.72 ml total

pooled frn 41-47  
(~10.7 ml total vol)

Dealyze into buffer F  
see P 115

Recovered 2.6 ml Tne after deanalysis (in by  
add 2.6 ml buffer G of 4-29-55 (sup 6, 10 ml 91342-1)

T Pag No

With ss d &amp; Understo d by m ,

J. Polansky

Date

7-24-55

Inv nt d by

R cord d by

Dat

7-22-95

7-22-95 24 0.67 ml/min  
Hepatic Tm

7-22-95

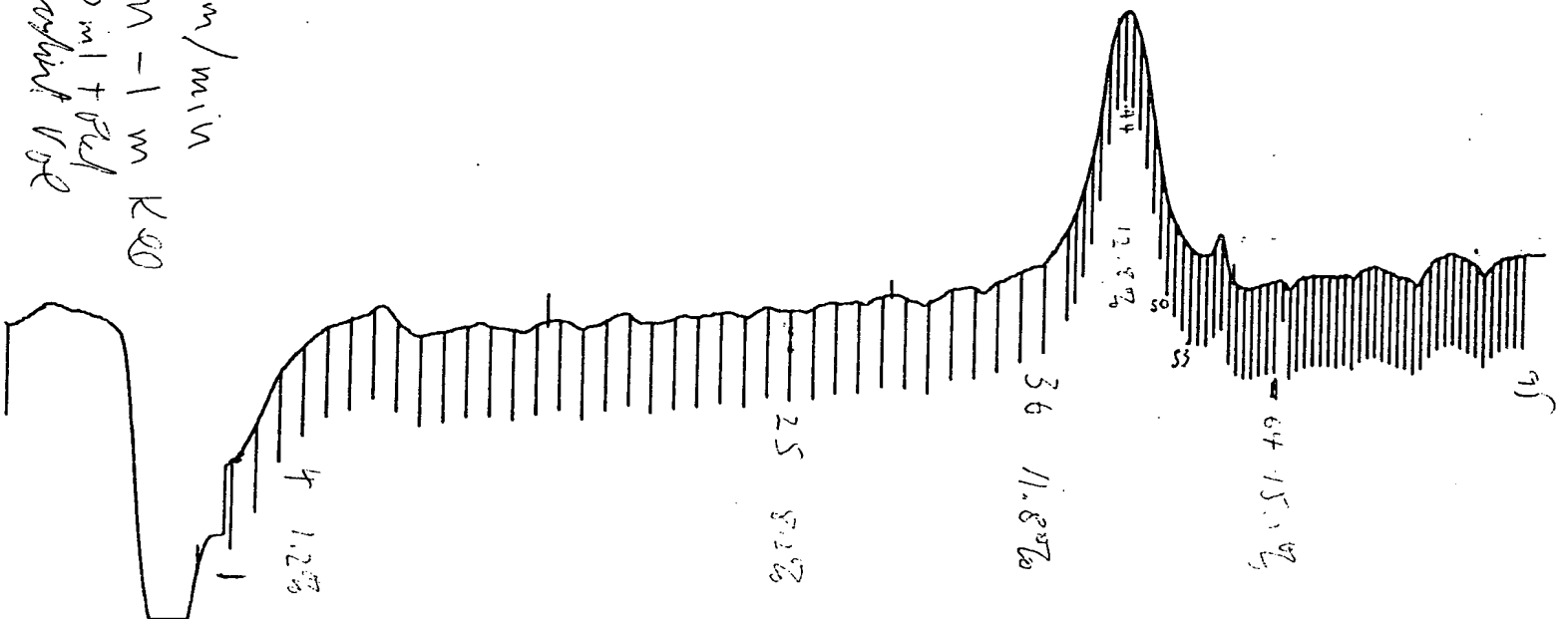
0.5 mm/min

50 mm - 1 m K20

650 ml + Red  
gradient 102

2 min  
fix

→



To Page No. \_\_\_\_\_

sed & Understood by me,

*Polany*

Date

7/24/95

Invented by

Recorded by

Date

7-22-95

Project No. \_\_\_\_\_

Exhibit L-139

118

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Appl. No. 09/558,421

From Page No. \_\_\_\_\_

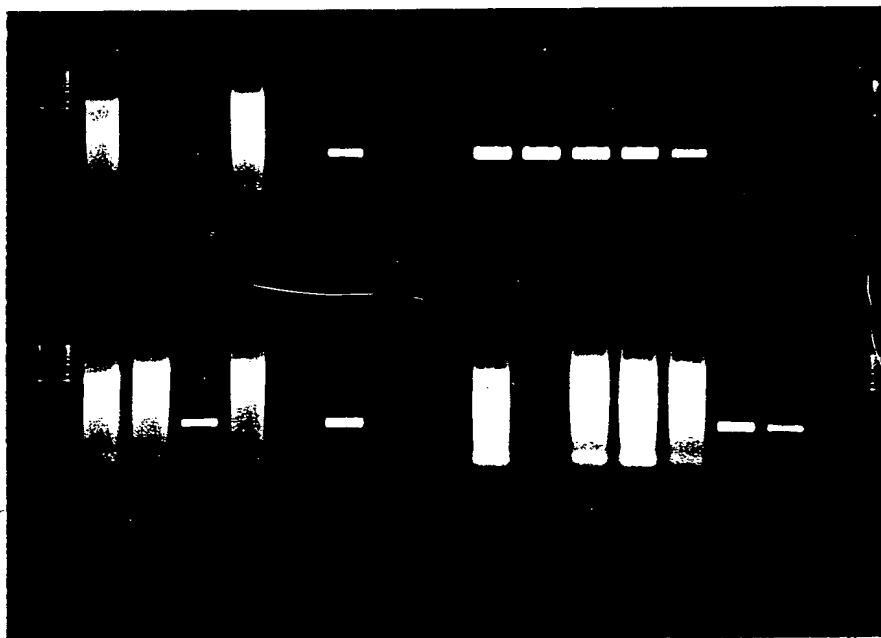
This experiment is detailed on p. 119-120, VB 11

The

Tag

J-7-95 L12

50ul rxns units 3 2.5 2.15 1 0.5 0.25 0.125



3 2.5 2.15 1 0.5 0.25 0.125

7-27-95

Blue  
Seph

Heparin

T Page 1

Witnessed & Und rsted by m ,

Dat

Inv nt d by

Dat

R corded by

Baron

PCR rxns with Blue Sepharose and Heparin  
fractionation of Tne prep 7/22/95

age N — 7/23/95

ation of Blue Sepharose pool fractions and Heparin pool fraction of Tne.  
Taq and Tne (5-7-95) prep will be tested in PCR alongside the new Tne.  
an we use more than 2 units of new Tne and not get a smear?  
ie 5-7-95 Tne prep gives a smear w/ more than 1 unit (p. 83 NB11)

tions:

Cheng buffer

15 cycles w/ old program ie lab 15 9600 #76 94°C 1min

est 0.125, 0.25, 0.5, 1, 1.5, 2, 2.5, 3 units

ake the 380bp product

50ul rxn

35x

94°C 30sec

55°C 30sec

72°C 2min

4°C

cocktail w/ all components except enzyme, for 34 rxns

340ul 5x Cheng

34ul 10mM dNTPs

34ul 50pg/ul M13 RF

34ul 20uM anchor primer > see p. 42 NB11

34ul 20uM 6681 primer

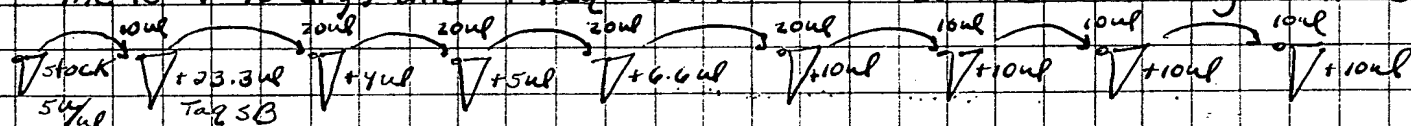
1156ul H<sub>2</sub>O house distilled

1632ul

→ 48ul / PCR tube for 9600

enzyme dilutions in Taq SB:

or Tne (5-7-95 lig) and rTaq - both 50ul do the following dilutions

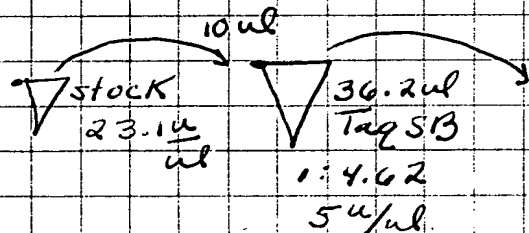


$D_1 = 1.5^4 \text{ul}$   $1.25^4 \text{ul}$   $1^4 \text{ul}$   $0.75^4 \text{ul}$   $0.5^4 \text{ul}$   $0.25^4 \text{ul}$   $0.125^4 \text{ul}$   $0.0625^4 \text{ul}$

start rxns w/ 2ul of each dilution on ice, flick, spin down

for Blue sepharose pool - 23.1  $\frac{\text{u}}{\text{ul}}$  when normalized to 5-7-95 Tne

ie.  $\frac{5}{7} (32.3) = 23.1$  p. 112, NB11



same as Tne (5-7-95) dilutions

To Page No. \_\_\_\_\_

Used & Understood by me,

Polamp

Date

8/1/95

Invented by

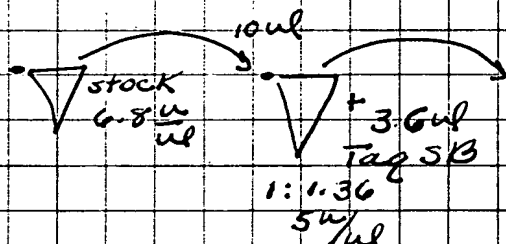
Recorded by Carolyn Comb

Date

7/27/95

From Page No. \_\_\_\_\_

Heparin fraction #44, which had peak UV absorbance  
 is  $6.8 \frac{\mu}{\mu l}$  when normalized to Tne (5-7-95 prep)  
 is  $\frac{5}{7} (9.53) = 6.8 \frac{\mu}{\mu l}$  p. 116 NB 11



same dilutions as for Tne (5-7-95) p. 11

start rxns w/  $2 \mu l$  of each dilution starting w/ the  
 $1.5 \frac{\mu}{\mu l}$  dilutions

35 cycles, stop w/ EDTA stop soln,  $7.5 \mu l$   
 run  $25 \mu l$  on gel

Result on p. 118 NB 11 - new Tne prep (7-22-95)  
 is not less prone to  
 making a smear than the  
 old (5-7-95) prep. So, DNA  
 contamination of enz should  
 not be Tne's main problem.  
 Rather, Tne may have an  
 intrinsic activity that makes  
 it "smear" more easily than  
 Tag

Witnessed & Understood by me,

Date

8/1/95

Investigated by

Recorded by

Phyllis Smith

Date

7/27/95

T Page 1



From Page No. \_\_\_\_\_

1) Can r-Taq make a smear in high  $Mg^{2+}$ ?  $\pm$  primers & temp.

mix A =  $\frac{494.4}{510.4 \text{ ul H}_2\text{O}}$   
 for 8 rxns  $\frac{80 \text{ ul } 10 \times \text{PCR buffer } \checkmark}{16 \text{ ul } 10 \text{ mM dNTP } \checkmark}$   
 $\frac{16 \text{ ul } 50 \text{ ng/ul m13 RF target } \checkmark}{16 \text{ ul } 20 \text{ uM anchor } \checkmark}$   
 $\frac{16 \text{ ul } 6681 \text{ primer, } 20 \text{ uM } \checkmark}{1.31 \text{ ul r-Taq } 8.28 \text{ u/ul } \checkmark}$   
 $\frac{1.29 \text{ ul H}_2\text{O}}{640 \text{ ul}}$

mix B =  $\frac{542.4 \text{ ul H}_2\text{O}}{8 \text{ rxns}}$   
 $\frac{80 \text{ ul } 10 \times \text{PCR buffer } \checkmark}{16 \text{ ul } 10 \text{ mM dNTP } \checkmark}$   
 $\frac{1.31 \text{ ul r-Taq } 5 \text{ u/ul } \checkmark}{1.29 \text{ ul H}_2\text{O } \checkmark}$   
 $\frac{640 \text{ ul}}{$

start rxns by adding  $Mg^{2+}$ 

	1	2	3	4	5	6	7	8	9	10	11	12	13
H <sub>2</sub> O	17.9	17	16	12	8	4	0	17.9	17	16	12	8	4

mix A  $\checkmark$   
 = + primers  
 template

80

mix B  $\checkmark$   
 no primers  
 no template

80

add last just before PCR  
 50 mM  $MgCl_2$   $\frac{2.1}{100 \text{ ul rxns}}$  3 4 8 12 16 20 2.1 3 4 8 12 16

35 cycles

stop whole rxn w/ 11 ul stop soln w/ 10x EDTA p 79  $\checkmark$ 

Witnessed &amp; Und rsto d by me,

Dat

Inv nted by

Dat

0001000

8/1/95

R cord d by

Paulm Pomb

7/27/95

T Pag N

g N \_\_\_\_\_

yes The work better (make more product) with a hot start?

mix C for 4.5 rxns = 90ul 5x Cheng ✓

301.5 ul H<sub>2</sub>O

9ul 50pg/ul m13 RF in TC from 2/94

9ul 20uM anchor primer ✓

9ul 20uM 6681 primer ✓

9ul 10mM dNTPS ✓

427.5

7/12/95

real 19.0 u/w

1:30.5x dilution

6:18.3

177ul Tag SB

95ul  
+ 5ul Tne 0.24ul

duplicate

hot

hot start

start

(15)

(16)

✓ cold start

duplicates

(17)

(18)

stop at 25, 30, 35 cycles

20ul  
+ 3ul stop

added eny  
after 11 94°C denaturation  
→ H5X 2

Can human spleen genomic DNA promote smear formation - bad seed?  
no template, no primers

high Mg<sup>2+</sup> - short smear  
2.5 rxns condition

Low Mg<sup>2+</sup> - long smear condition

25ul 10x PCR buffer ✓

187.5ul H<sub>2</sub>O ✓

5ul 10mM dNTPS ✓

7.5ul 50mM MgCl<sub>2</sub> ✓

225ul

90ul D ✓

2.5ul H<sub>2</sub>O ✓

2.5ul genomic DNA ✓

5ul Tne 0.24ul

(19)

90ul D ✓

5ul H<sub>2</sub>O ✓

5ul Tne

(20)

25ul 10x PCR buffer ✓

189.75ul H<sub>2</sub>O ✓

5ul 10mM dNTPS ✓

5.25ul 50mM MgCl<sub>2</sub> ✓

225ul

90ul E ✓

2.5ul H<sub>2</sub>O ✓

2.5ul genomic DNA ✓

5ul Tne

(21)

90ul E ✓

5ul H<sub>2</sub>O ✓

5ul Tne

(22)

remove 10ul at 15, 20, 25, 30, 35 cycles STOP tubes + 2ul stop To Page No. \_\_\_\_\_

sed & Understood by me,

Polamp

Date

8/1/95

Invented by

Recorded by

Date

7/27/95

From Page No. \_\_\_\_\_

4) Does Tne make a smear when 1 or 2 dNTPs are missing from rxn?

4 dNTP mix, 2.5 mM each  $\Rightarrow$  20  $\mu$ l each 10 mM dNTP stock

3 dNTP mix, AGC 2.5 mM each  $\Rightarrow$  20  $\mu$ l 10 mM A ✓

20  $\mu$ l 10 mM G ✓

20  $\mu$ l 10 mM C ✓

20  $\mu$ l H<sub>2</sub>O ✓

use  
8  $\mu$ l of  
mixes  
each 100  
PCR rxn  
for CF=2

2 dNTP mix, GT 2.5 mM each  $\Rightarrow$  20  $\mu$ l 10 mM G ✓

20  $\mu$ l 10 mM T ✓

40  $\mu$ l H<sub>2</sub>O ✓

mix F = 200  $\mu$ l 10x PCR buffer ✓  
for 20 rxns  
1480  $\mu$ l H<sub>2</sub>O ✓  
1680  $\mu$ l

for 9.5 rxns

[G] 798  $\mu$ l ✓  
28.5  $\mu$ l 50 mM MgCl<sub>2</sub> ✓  
883.5  $\mu$ l

[H] 798  $\mu$ l ✓  
19.95  $\mu$ l 50 mM M ✓  
8.55  $\mu$ l H<sub>2</sub>O ✓  
883.5  $\mu$ l

20  $\mu$ l G 20  $\mu$ l G K 20  $\mu$ l  
24  $\mu$ l 4 dNTP 24  $\mu$ l 3 dNTP mix  
24  $\mu$ l 2 dNTP

695  $\mu$ l  
+ 5  $\mu$ l Tne. 24  $\mu$ l

23, 24 25, 26 27, 28

100  $\mu$ l 35 cycles  
11  $\mu$ l stop (p. 79)

Lab 16, 9600 method 103 1:25<sup>PM</sup>

T Page N

With ss d &amp; Und rst d by me,

Q601cung

Dat

8/1/95

Inv nt d by

R cord d by

Danish Pomb

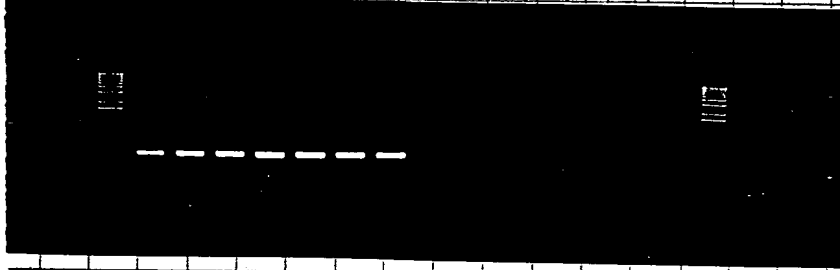
Dat

7/27/95

ag N \_\_\_\_\_

2% agarose gel  $\Rightarrow$  3.6g agarose  
 300mL 1X TAE  
 20uL EtBr 5989

target + primers  $\xrightarrow{\text{Taq}}$  no input DNA  
 NgCl<sub>2</sub> 1.05 1.5 2 4 6 8 10 1.05 1.5 2 4 6 8 10



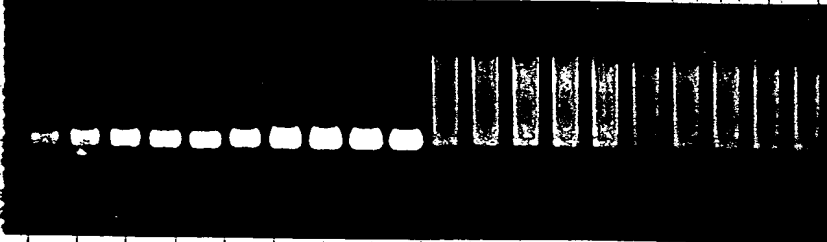
C. Comb  
 7/25/95

cycles  
 25 30 35 25 30 35 25 30 35 25 30 35  
 Tne (7-22-95 prep)  $\rightarrow$  Cheng buffer  
 hot start cold start primers + target  
 3uL/rxn



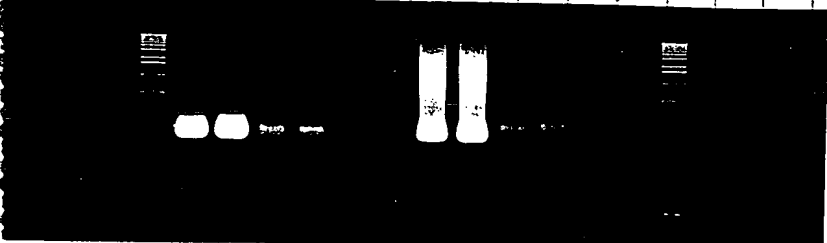
C. Comb  
 7/28/95

1.5 mM MgCl<sub>2</sub> Tne (7-22-95) 1.05 mM MgCl<sub>2</sub>  
 + genomic - genomic + genomic - genomic  
 15 20 25 30 35 15 20 25 30 35 15 20 25 30 35 15 20 25 30 35  
 PCR buffer 3uL/rxn  $\Rightarrow$  too much



C. Comb  
 7/28/95

present  
 1.5 mM MgCl<sub>2</sub> Tne (7-22-95) 1.05 mM MgCl<sub>2</sub>  $\rightarrow$  PCR buffer  
 4 4 3 3 2 2 4 4 3 3 2 2 35 cycles  
 3uL/rxn



C. Comb  
 7/28/95

top  
 1-14  $\Rightarrow$  25uL  
 15-18 12 tubes 20uL inc. cycles  
 20 tubes 19-22  $\Rightarrow$  10uL inc. cycles  
 23-34 10 19 15C 19 20C 19 25C  
 5 25uL ect

bottom

### Results

Increasing Mg<sup>2+</sup> did not cause Taq to make a smear either in the presence or absence of input DNA

With 3 units Tne, the hot start rxns did not smear any less than cold start rxn. I should have used 1.36 units in order to get product instead of smear redo this expt w/ 1.36 units Tne rxn

Addition of genomic DNA did not result in less smear over time w/ 3 units Tne

redo this expt w/ 1.36 units Tne rxn

3 dNTP mix = AGC present  
 2 dNTP mix = GT present

No smear made when 2 dNTPs (C+A) are missing, so smear is probably not made by a TdT activity. To Page No. \_\_\_\_\_

and Und rstood by me,

Polamp

Date

8/1/95

Invented by

Recorded by

Carolyn Comb

Date

7/27/95

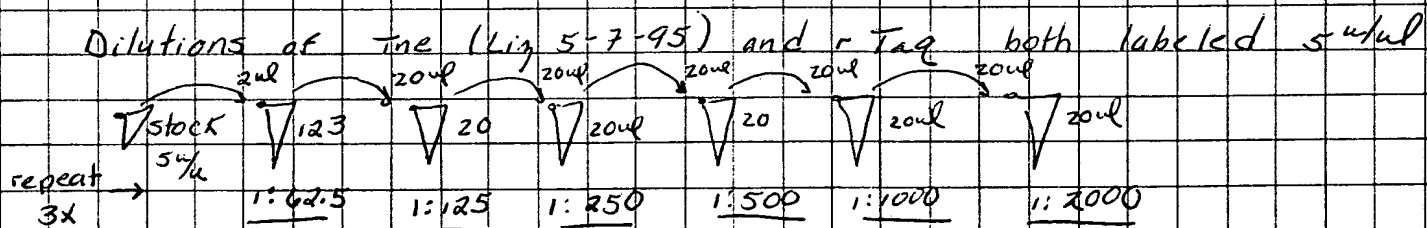
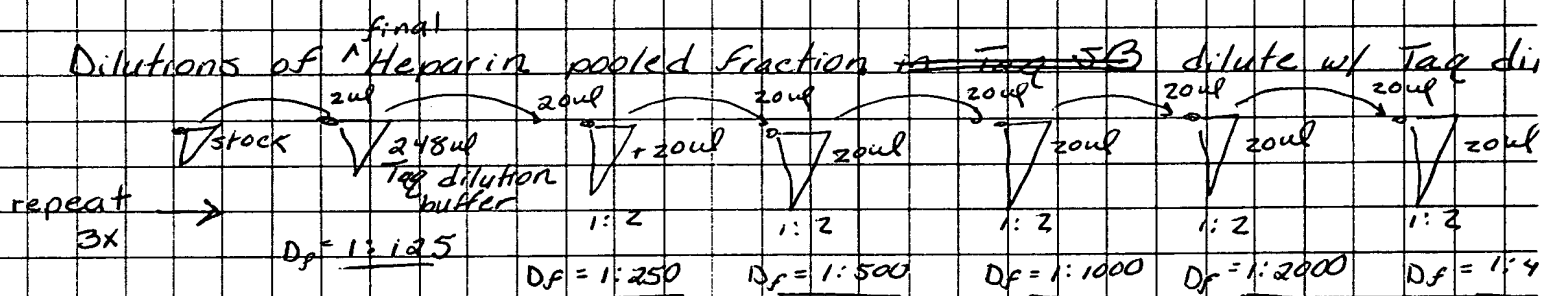
From Page No. 7/20/95 Unit assay of new Tne prep (7-22-95) after dialysis into Tag storage buffer.

- old 5-7-95 Tne prep & r Tag will be done too
- 3 replicates of each dilution series, done 3x independent

cocktail: 200  $\mu$ l of Taps,  $MgCl_2$ , KCl mix -  
 2,520  $\mu$ l  $H_2O$  w/ p1000  
 60  $\mu$ l 10mM dNTPs  
 405  $\mu$ l 3.7 mg/ml gapped DNA  
 9.3  $\mu$ l  $^{32}P$  dCTP ref date 7/14/95  
3.2 mL

use 48  $\mu$ l per rxn

start rxns by adding 2  $\mu$ l of enzyme dilutions on ice.



- rxns stopped w/ 10  $\mu$ l 0.5M EDTA
- 20  $\mu$ l of each rxn spotted on GFC filters

SAM CPM1 ave pmol ave u/ul C. Combs 7/27/95

Project No. \_\_\_\_\_  
B ok No. \_\_\_\_\_

7-22-95

1	8081.00		
2	7346.00	625	11.7
3	5871.00		
4	5139.00		
5	5341.00	441	16.5
6	4559.00		
7	3009.00		
8	2963.00	255	19.1
9	2724.00		
10	1492.00		
11	1359.00	125	18.8
12	1429.00		
13	891.00		
14	899.00	79	
15	895.00		
16	490.00		
17	402.00		
18	524.00		

The 7-22-95  
19.0 u/ave

⇒ use (x ul)(19.0) = 1.36 u/100ul  
⇒ 0.0716 ul/100ul PCR rxn

7-22-95

19	6160.00		
20	6476.00	582	5.4
21	7195.00		
22	4215.00		
23	4266.00	354	6.6
24	3596.00		
7	2124.00		
8	2014.00	181	6.8
9	2055.00		
10	1160.00		
11	998.00	93	7.0
12	1024.00		
13	572.00		
14	610.00		
15	609.00		
16	361.00		
17	352.00		
18	348.00		

The Liz  
6.8 u/ave

⇒ we had been using 0.2 ul/100ul rxn  
0.2 ul is really 1.36 units, not unit  
as we thought based on the  
unit value 5 u/ul

7-22-95

19	8453.00		
20	6925.00	658	6.17
21	7075.00		
22	4769.00		
23	3803.00	387	7.25
24	4613.00		
25	2896.00		
26	2565.00	240	9.00
27	2722.00		
28	1185.00		
29	1404.00	115	8.60
30	1334.00		
31	1234.00		
32	873.00	89.7	13.40
33	953.00		
34	592.00		
35	527.00		
36	509.00		

Tag  
ave 8.28

⇒ use (x ul)(8.28 u/ul) = 1.36 u/100ul PCR  
= 0.164 ul/100ul Tag

↑ expected 5 u/100ul  
u/100ul  
real units in n mol/30'

7-22-95

37	56483.00		
38	57656.00		
39	56427.00	56855 = 34.1 CPM/pmol	

To Page No. \_\_\_\_\_

& Understood by me, Polamp	Date 8/1/95	Invent d by 	Date 7/27/95
		Recorded by Carolyn Combs	

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

JU containing templates  
(Myron Goodman assay)

From Page No. \_\_\_\_\_

Exhibit L-141

Appl. No. 09/558,421

"Fidel pri" (27mer)  
7-11-95

CGAGACATGGCGTCCAGTCACGACCT  
GCTCTGTACCGCAGGGTCAGTGCTGGACTAGTACGAGCTACT

27 bp


"Fidel Template"  
or "Fidel Template"  
(7-11-95)  
(42mer)


#51351

This is not old "Fidel Template"  
of 1951. This new one  
less stringent for primer  
according to oligo program

1. ssDNA region is same as MB JBC Criggleton & MG R  
for + dGTP + dATP (get G-A mismatch at position 3 and)  
+ dCTP for reverse

2. For test of dATP incorp opposite Template JU,  
have all 4 dNTPs present at 200  $\mu$ M each  
and look for pause one site before JU  
(run on 7th PAGE?)

G  
C  
A ↑  
U T A  
G C (31)   
A T  
T A  
C G

primer (27) 

To Page No.

Witnessed &amp; Understood by me,

Date

Invented by

Date

Deanna D. S. S.

8/1/95

Recorded by

7-28-95

JU vs JT in template

From Page N \_\_\_\_\_

① ② ③ ④ ⑤ ⑥ ⑦ ⑧

<sup>32</sup>P pri. ofide / Temp JT  
50 mM primer (PST)

4 →

10 mM

<sup>32</sup>P pri. ofide / Temp JU  
250 mM primer (PST)

4 →

~ 10 mM  
total10X Tag PCR buffer  
50 mM MgCl<sub>2</sub>10 10  
3 310 10  
3 31.5 mM  
Cl<sup>-</sup>

10X Vent buffer

10 10

10 10

10 mM dNTPs

2 μl →

2.5 mM  
dNTP

rTag 0.0625 μM

2

2

Tne 0.0625 μM

2

2

0.125 μM  
2 to

Vent 0.0625 μM

2

2

0.013 μM  
pol m

DeepVent 0.0625

2

2

0.013 μM  
pol mH<sub>2</sub>O

79 →

72 →

79 →

72 →

0.013 μM  
pol m

preheat to 70°C

100 μl

Start with addition of pol.

so has  
primer  
overpr

remove 10 μl to 5 μl cycle seq stop at

0 5 10 20 40 60 90

To Page No \_\_\_\_\_

Witnessed &amp; Understood by me,

D. D. Camp

Date

8/1/95

Initiated by

R. C. R. d. by

Date

7-27-95



age N \_\_\_\_\_

sequence Rxn same as P 27, 4 and 90 11  
 using <sup>32</sup>P pri fidel Temp with ST und du

To Page No. \_\_\_\_\_

s d & Understood by me,

*Polkamp*

Date

8/1/95

Invented by

Recorded by

Dat

7-27-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Effect of annealing temperature on the smear

128

From Page No. \_\_\_\_\_

- anneal at 50°C, 60°C, 70°C w/ 72°C extension - no input DNA
- take out rxn aliquots after 10, 15, 20, 25, 30, 35 cycles
- use 1.36 units Tne (5-7-95 Lig) / 100ul rxn
- test the effect of annealing temp on both the long (low Mg<sup>2+</sup>) and short (hi Mg<sup>2+</sup>) smears

mix A = high Mg<sup>2+</sup> for small smear - enough for 3.5 rxns

280ul H<sub>2</sub>O ✓  
35ul 10x PCR buffer ✓  
7ul 10mM dNTPs C<sub>f</sub> = 200uM ✓  
10.5ul 50mM MgCl<sub>2</sub> C<sub>f</sub> = 1.5mM ✓  
332.5ul

mix B = low Mg<sup>2+</sup> for long smear - enough for 3.5 rxn

283.15ul H<sub>2</sub>O  
35ul 10x PCR buffer ✓  
7ul 10mM dNTPs  
7.35ul 50mM MgCl<sub>2</sub> / C<sub>f</sub> = 1.05mM  
332.5ul

\* 25 fold dilution  
The stock (Lig: 50ul (real units p. 127)  
3ul Tne  
72ul Tag  
75ul of o.

annealing temp	50°C Lab 15		60°C Lab 16		70°C Lab 562		72°C Tag 75 ul of 0.
rxn #	1	2	3	4	5	6	← 5 & 6 received mix that were made up a different time to the mix A & B for 1
1 ul Mix A hi mg	95		95		95		
1 ul Mix B low mg		95		95		95	
* ul Time 0.24ul 5-7-95 Lig	5	—————→					
	100ul rxns						

596 received mix that were made up a different time to the mix A+B for 1

remove 10ul of rxn to 2ul STOP soln w/ EDTA p. 79 NB11  
at cycles 10, 15, 20, 25, 30, 35

T Page 1

Witnessed & Und rst d by m ,

Dat

Invent d by

Dat

DB Olamp

8/1/95

R corded by

Daniel Poms

7/28/95

ag N. \_\_\_\_\_

cling Lab 15 program 76 94° 1min  
 35x { 94° 30sec  
 50° 30sec  
 72° 2min  
 4° —

Lab 16 program 103 94° 1min  
 4.5 min per cycle 35x { 94° 30sec  
 60° 30sec  
 72° 2min  
 4° —

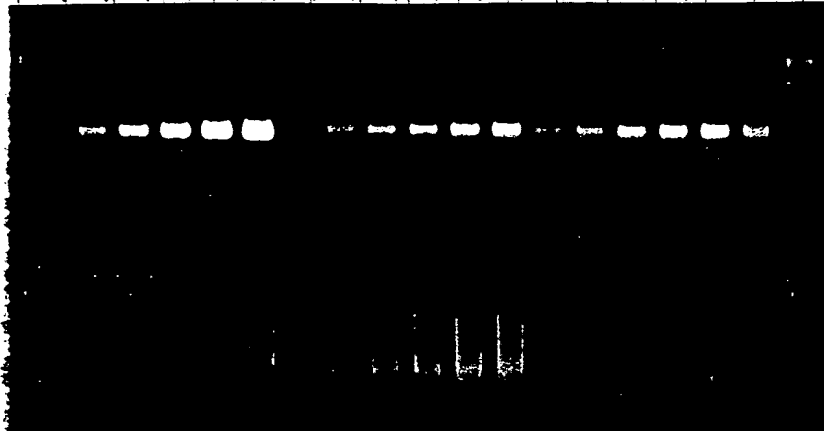
SG1 program 133 94° 1min  
 94° 30sec

2 temp. PCR 35x { 94° 30sec  
 72° 2min 30sec  
 4° —

2% agarose gel, 36 samples + primers { 2ul 200mM NaCl  
 2ul 200mM 6681  
 9ul H<sub>2</sub>O  
 11ul stop  
 load 20ul  
 + uncycled Tne { 1.5ul Tne Lig  
 30ul 503  
 65ul  
 95ul H<sub>2</sub>O  
 11ul stop  
 load 20ul  
 12 short 50°  
 31-10-10-35; 3-10-10-35; 5-10-10-35;  
 10 long 50°  
 2-10-10-35; 4-10-10-35; 6-10-10-35;  
 primers, 7/5/95 Tne, 7/22/95 Tne  
 2ul 200mM NaCl  
 2ul 200mM 6681  
 9ul H<sub>2</sub>O  
 11ul stop  
 load 20ul  
 primers alone, 8 samples and 8 samples 6681  
 5-7-95 Tne  
 7-22-95 uncycled

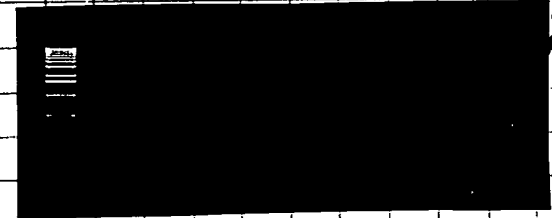
Tne (5-7-95 Lig) 1.3644 min

ing 50°C 60°C 72°C  
 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35



1.5mM  
MgCl<sub>2</sub>

1.05mM  
MgCl<sub>2</sub>



Comb  
128/95

To Page No. \_\_\_\_\_

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Cathy Comb

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7/28/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE *unit assay for 1.1X Tag*  
*same as P121, 9 and P52, 10*

From Page No. \_\_\_\_\_

From Page No.	Rxn#	ul/assay	1% Tween 20/NP40 vol
4°C #10 (P121, 9) (no det)	1-3	2	1 ul
11 1.1X	4-6	3.64	
1 (2X 0.1% TN)	7-9	2	
14 (2X TFI buffer)	10-12	2	
rTag (same as P121, 9) K5 dil	13-17	2	
<del>From Temp</del> 1.1X called	18-20	2	
"new" on P34 (58.95)			
From Temp #11 1.1X	21-23	2.64	
-20 1-27-95	24-26	2	
5 months on P52			
(see Rxn# 21-23 on P52)			
got 5-4% recovery (on P53)			

Exhibit L-143

Appl. No. 09/558,421

°C	SAM	CPM1	ave	ul/ul	% of zero time P122, 9
10	1	1779.00			
	2	1970.00	2029	.008	38%
	3	2337.00			
11	4	8375.00		.033	103
	5	8284.00			
	6	8267.00			
1	7	10246.00	9774	.039	105
	8	9851.00			
	9	9556.00			
14	10	8959.00			
	11	9908.00	9484	.037	107
	12	9584.00			
rTag	13	10530.00			
	14	9527.00	10,119	(.04 by definition)	
	15	9706.00			
	16	9859.00			
	17	10773.00			
20	18	6924.00	7063	.028	(was .025 on P53 so >100%)
25	19	7046.00			
	20	7219.00			
1	21	6156.00			
	22	6520.00	6257	.025	77% (P154, 9 is 0 time po (P38, 10))
	23	6095.00			
20°C	24	5038.00			
25	25	4980.00		.019	$\frac{.017}{.030} = 63\%$ recovered see P53
27.5	26	4755.00			
30	27	82.00			
rel	28	119284.00			
	29	121726.00			

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Hot vs Cold start PCR w/ Tne (5-

130

From Page No. — Is more <sup>specific</sup> product made and less smear in a hot vs cold start  
 Look at products at 10, 15, 20, 25, 30, 35 cycles

• start 100ul rxns w/ 2ul enz in order to keep [glycerol] low  $\Rightarrow$  SB + 50%, rxns will be 1%, do duplicate hot + cold start rxns

• materials: ~~5x stock of Tne~~

[A] mix: 100mM Tricine pH 9  $\Rightarrow$  100ul 1M Tricine pH 9 ✓  
 5.25mM MgOAc 5.25ul 1M MgOAc ✓  
 4.25mM KOAc 212.5ul 2M KOAc ✓  
 682.25ul H<sub>2</sub>O ✓  
 1mL

cocktail for 4.5 reactions = 90ul 5x [A] mix ✓  
 315ul H<sub>2</sub>O ✓

[B]  
 9ul 50pg/ul M13RF ✓  
 9ul 20uM anchor primer ✓  
 9ul 20uM 6681 primer ✓  
 9ul 10mM dNTPs ✓

441ul

98ul B 98ul B 98ul B 98ul B  
 2ul Tne 0.684ul 2ul Tne 2ul Tne 2ul Tne

①

②

③

④

cold start

hot start - em a  
 2:40 PM denati  
 of 1st

Tne dilution: 3ul Tne (5-7-95 Lig) 0.8uM ✓  
 27ul Tne SB ✓

30ul of 0.684uM Tne

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Bobolamp

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Paulen Pomb

ag N \_\_\_\_\_

move 10ul after 10, 15, 20, 25, 30, 35 cycles + add 2ul STOP w/ 100mM EDTA  
 ab 14 9600 program 10.3 = 94°C 1min

35x { 94°C 30sec  
 55°C 30sec  
 72°C 2min  
 4°C \_\_\_\_\_

2% agarose gel

top

→ 1-10C 10 1-35C 4-35C } 2  
 25 19-10C 22-35C } 2

lit:

Tne (5-7-95 Liz prep) 1.36 w/rxn

Cold Start - duplicates

Hot Start - duplicates

cycle#

10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35

buffer = 20mM Tricine

85mM KOAc

1.05mM MgCl<sub>2</sub>3146  
2C

- 380bp m.3 product

conclusion: Hot start did not result in more specific product.  
 One cold start duplicate rxn failed, no apparent reason.

To Page No. \_\_\_\_\_

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Paulyn Cumb

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From Page No. 123

same as p. 123 x3 except only 1.36  $\mu$ l of Tne (5-7-95)  
will be used per rxn

x 19 90  $\mu$ l D ✓  
5.5  $\mu$ l H<sub>2</sub>O ✓  
2.5  $\mu$ l genomic DNA <sup>5/6/95</sup> ASX2 ✓  
2  $\mu$ l Tne 0.68  $\mu$ l p.130

x 20 90  $\mu$ l D ✓  
8  $\mu$ l H<sub>2</sub>O ✓  
2  $\mu$ l Tne

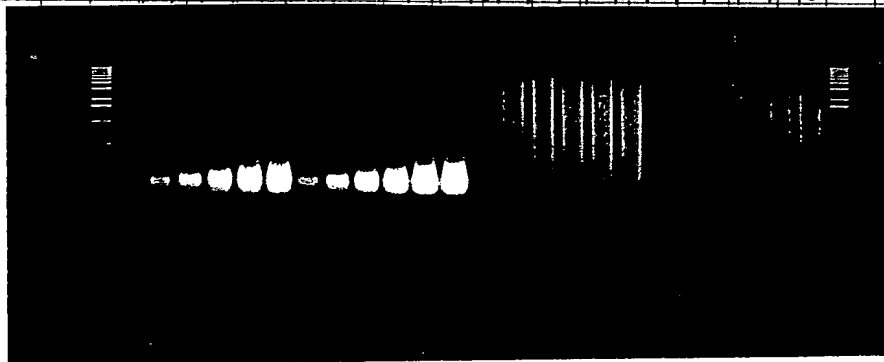
x 21 90  $\mu$ l E  
5.5  $\mu$ l H<sub>2</sub>O  
2.5  $\mu$ l g  
2  $\mu$ l Tne

x 22 90  $\mu$ l E ✓  
8  $\mu$ l H<sub>2</sub>O ✓  
2  $\mu$ l Tne

10  $\mu$ l aliquots removed after 10, 15, 20, 25, 30, 35 cycles + 2  $\mu$ l stop w/ 100 mM  
Tne 1.36  $\mu$ l/rxn (5-7-95 Lig prep) - no pc  
prese.

Result:

1.5 mM MgCl<sub>2</sub> + genomic - genomic  
1.05 mM MgCl<sub>2</sub> + genomic - genomic  
cycle x → 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35

7/31/95<sup>cc</sup>

Conclusion: 1) The smear is visible even after 10 cycles - we should  
run aliquots of earlier cycles to determine when the  
smear becomes visible.

2) The 1.05 mM Mg<sup>2+</sup> long smears are more intense with  
genomic DNA present than without genomic DNA. T  
may be a real result or it may be variation in rxns - repeat w/ triplicates

bit of support for  
idea that genomic  
DNA can act as  
a bad seed.

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7/31/95

D. Polansky

Paulen Pomb

30 Pag N

[Mg<sup>2+</sup>] titration in a short PCR rxn w/ Tne

(5/7/95 Liz)

Page No. \_\_\_\_\_

pose: To demonstrate that lowering [Mg<sup>2+</sup>] shifts the size of DNA products in smear from small to longer ~~in~~ in a short PCR rxn w/ Tne alone. This result has been observed w/ ~~the~~ 1.5mM Mg<sup>2+</sup> vs 1.05mM Mg<sup>2+</sup>, but the range of concentrations has not been tested. Does the transition occur over a narrow or broad range of Mg<sup>2+</sup>? What happens w/ 85mM KOAc + 1.5mM Mg<sup>2+</sup>? We have not tested this condition before

real expt conditions:

Cf MgOAc (mM) 0.9, 1.05, 1.1, 1.15, 1.2, 1.3, 1.4, 1.5, 1.7 (9 levels)  
Cf KOAc (mM) 50, 85 (2 levels)

template and primers for m13 380bp product present  
35 cycles, 55°C annealing temp

1.36 u/round rxn Tne - 5-7-95 Liz prep

start rxns w/ Mg<sup>2+</sup>

materials: 25mM MgOAc →  $\frac{25\text{mM } 100\mu\text{L}}{1000\text{mM}} = 25\mu\text{L } 1\text{M MgOAc}$   
975 uL H<sub>2</sub>O

A - 50mM KOAc for 9.5 rxns

mix B = 85mM KOAc 9.5 rxns

19 uL 1M Tricine pH 9.0 ✓

23.75 uL 2M KOAc ✓

734.35 uL H<sub>2</sub>O ✓

19 uL 10mM dNTPs Cf = 200 uM ✓

19 uL 20 uM 6681 primer ✓

19 uL 20 uM anchor primer ✓

19 uL 50% m13 RF ✓

1.9 uL Tne (5-7-95 Liz prep) = 1.36 u/rxn

855 uL  
6.8 u/rxn

19 uL 1M Tricine pH 9 ✓

40.375 uL 2M KOAc ✓

717.725 uL H<sub>2</sub>O ✓

19 uL 10mM dNTP ✓

19 uL 20 uM 6681 ✓

19 uL 20 uM anchor ✓

19 uL 50% m13 ✓

1.9 uL Tne 5-7-95

855

top of gel

1 2 3 4 5 6 7 8 9 10-18

no tube 9 - I dropped it

there is a tube 18

12 PM - 1:30 PM

Lab 16 9600 10.3

90 uL — 1

90 uL — 1

6.4 5.8 5.6 5.4 5.2 4.8 4.4 4 3.2 same series as 1-9

3.6 4.2 4.4 4.6 4.8 5.2 5.6 6 6.8

add Mg, mix well & keep on ice til cycling

100 uL rxns

stop w/ 100mM EDTA, run 20 uL on 1.2% agarose gel

To Page No. \_\_\_\_\_

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Date

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Date

Research Policy

8/7/95

Recorded by

8/1/95



# Addition of genomic DNA to a Tne short PCR rxn - 3, 6, 10, 12, 15, 20 cycle aliquot

ag N.132

urpose: To determine if adding human genomic DNA to a Tne PCR rxn leads to production of a smear by an earlier cycle # than without genomic DNA. Genomic DNA <sup>200ng/100ul rxn</sup> might act like more "bad seed" material and exacerbate the smearing rxn. This experiment was tried on p. 132. Cycle #'s 10-35 were run on the gel. There was an indication that addition of genomic DNA made the smear darker by an earlier cycle #. Now, we are repeating the p.132 exp in triplicate and looking at even earlier cycle #s.

Summary of experimental cond: 3 rxns w/ human spleen DNA, 3 without  
materials: no primers added

start rxns by adding 1.36ul/100ul rxn w/  
Tne (5-7-95 Lig prep)

55°C annealing temp program 103, Lab 10  
200ng of human spleen DNA/rxn

materials: mix A: for 7.5 reactions = 75ul 10x PCR buffer  
569.25ul H<sub>2</sub>O  
15ul 10mM dNTPs  
15.75ul 50mM MgCl<sub>2</sub>  
Tne (5-7-95 dilution  
2ul Tne  
15ul 5.0  
20ul 0.68  
Tne

	top of gel				bottom of gel		
	1	2	3		4	5	6
A	90ul	—	—	1	—	—	—
1	5.5ul	—	—	1	8ul	—	—
2	2.5ul	—	—	1	none	—	—
3	2.5ul	—	—	1	—	—	—
4	2.5ul	—	—	1	—	—	—
5	2.5ul	—	—	1	—	—	—
6	2.5ul	—	—	1	—	—	—
7	2.5ul	—	—	1	—	—	—
8	2.5ul	—	—	1	—	—	—
9	2.5ul	—	—	1	—	—	—
10	2.5ul	—	—	1	—	—	—
11	2.5ul	—	—	1	—	—	—
12	2.5ul	—	—	1	—	—	—
13	2.5ul	—	—	1	—	—	—
14	2.5ul	—	—	1	—	—	—
15	2.5ul	—	—	1	—	—	—
16	2.5ul	—	—	1	—	—	—
17	2.5ul	—	—	1	—	—	—
18	2.5ul	—	—	1	—	—	—
19	2.5ul	—	—	1	—	—	—
20	2.5ul	—	—	1	—	—	—
21	2.5ul	—	—	1	—	—	—
22	2.5ul	—	—	1	—	—	—
23	2.5ul	—	—	1	—	—	—
24	2.5ul	—	—	1	—	—	—
25	2.5ul	—	—	1	—	—	—
26	2.5ul	—	—	1	—	—	—
27	2.5ul	—	—	1	—	—	—
28	2.5ul	—	—	1	—	—	—
29	2.5ul	—	—	1	—	—	—
30	2.5ul	—	—	1	—	—	—
31	2.5ul	—	—	1	—	—	—
32	2.5ul	—	—	1	—	—	—
33	2.5ul	—	—	1	—	—	—
34	2.5ul	—	—	1	—	—	—
35	2.5ul	—	—	1	—	—	—
36	2.5ul	—	—	1	—	—	—
37	2.5ul	—	—	1	—	—	—
38	2.5ul	—	—	1	—	—	—
39	2.5ul	—	—	1	—	—	—
40	2.5ul	—	—	1	—	—	—
41	2.5ul	—	—	1	—	—	—
42	2.5ul	—	—	1	—	—	—
43	2.5ul	—	—	1	—	—	—
44	2.5ul	—	—	1	—	—	—
45	2.5ul	—	—	1	—	—	—
46	2.5ul	—	—	1	—	—	—
47	2.5ul	—	—	1	—	—	—
48	2.5ul	—	—	1	—	—	—
49	2.5ul	—	—	1	—	—	—
50	2.5ul	—	—	1	—	—	—

move 10ul to a tube on ice w/ 2ul STOP soln in it (100mM EDTA)  
3, 6, 10, 12, 15, 20 cycles, run 10ul on 1.2% agarose gel  
6 + 6-9: ~3ul 6-9 went into 6-6C stop tube c=cycle #  
See result on p. 134

To Page No. 134

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Date

elected a Polym

8/1/95

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8/1/95

Cawlyn Combs

From Page No. \_\_\_\_\_

Tne (5-7-95 Lig prep) 1.36  $\mu$ /rnn, 100ul rnn w/ template + primers

MgOAc (mM) 0.9 | 1.0 | 1.1 | 1.5 | 1.2 | 1.3 | 1.4 | 1.5

50 mM  
KOAc85 mM  
KOAc

• 1.3 mM MgOAc was optimal for making product - note that we have made pre w/ 1.05 mM  $Mg^{2+}$  in earlier e when glycerol + ~~1.05~~ also pre. p. 86

• 50 mM KOAc is not sufficient for product formation, but 85 mM KOAc is - values between 50 + 85 have not been tested

• The size of DNA products in sme does vary from small to longer as  $[Mg^{2+}]$  varies from 1.5 - 0.9 mM Mg

triplicate rxns w/ Tne (5-7-95 Lig prep)

(see P135 for reaction)

replicate 1 replicate 2 replicate 3

cycle # 3 | 6 | 10 | 12 | 15 | 20 | 3 | 6 | 10 | 12 | 15 | 20 | 3 | 6 | 10 | 12 | 15 | 20

Conclusion.

+ 200 ng human  
spleen genomic  
DNA per 100ul rxn

There maybe a 1x  
amount of contam.  
DNA still (or RNA  
in TNE prep -  
3-4 cycles needed  
see smear

no genomic DNA

e. emb  
3/2/95

T Page 1

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8-2-95

From Page No. \_\_\_\_\_

purpose: To determine if Mg<sup>2+</sup> controls Long PCR smear size and to see if the smear is primer/template independent. Note that Long PCR rxn uses different buffering components than we've been using for st.

In a single Tne enz PCR of 380 bp product, 1.2 mM Mg<sup>2+</sup> - 1.3 mM is at the center of transition from small to long smear is optimal for product formation if 85 mM KOAc present.

do: 7 levels of Mg<sup>2+</sup>, ± target and primers, 2 ratios of Tag: Tne

materials: LTI's Tag Long PCR system + Kalas recommendations for 7-22-95 Tne prep

dilution of Tne in Tag SIB: 2 ul Tne (7-22-95 prep, 19<sup>u</sup>/ul)

① 59.64 ul Tag SIB ✓ 1:30.82  
mix

② 2 ul of dilution ①  
59.64 ul Tag SIB 1:30.82

61.64 ul of 0.02<sup>u</sup>/ul Tne

enzyme mixes:

Final Tag (u)	Tne (mM)	Tag (5 <sup>u</sup> /ul)	Tne (0.02 <sup>u</sup> /ul)	SIB
1	1	4 10	1 2.5	15 3
1	10	4 10	10 25	8 1
1	2	2 5	4 2.5	7 1

mix 1 with primer and template for 16 rxns: 14 ul 10 mM dNTP  
14 ul primer mix 1  
156 ul DNA 1  
2 mg/ml  
3 ul total genomic temp  
27.2 ul H<sub>2</sub>O  
320 ul

mix 1 without primer + template, 16 rxns:  
14 ul 10 mM dNTP  
304 ul H<sub>2</sub>O  
320 ul

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Dancer Backup

8/12/95

ag No. _____	Mg <sup>2+</sup> mM	
2 (1:1 TT) 1 for 5 rxns =	50	✓
122: Tne	50 ul 5x A	25 ul
	0 & 30 ul 5x B	
	5, 5 ul 1u Tag: 1mU Tne	(7-22-95 prep)
	95 (95 ul H <sub>2</sub> O)	
	150	
2 (1:1 TT) 1.1 =	45 ul 5x A ✓	
mM Mg <sup>2+</sup>	5 ul 5x B ✓	
	5 ul 1u Tag: 1mU Tne	→ (200 ul H <sub>2</sub> O eng mix)
add H <sub>2</sub> O	95 ul H <sub>2</sub> O	→ 200 ul
2 (1:1 TT) 1.2 mM Mg <sup>2+</sup> =	40 ul 5x A ✓	
	10 ul 5x B ✓	→ Barry's aliquots
	5 ul 1u Tag: 1mU Tne	
	95 ul H <sub>2</sub> O	
2 (1:1 TT) 1.3 mM Mg <sup>2+</sup> =	35 ul 5x A ✓	
	15 ul 5x B ✓	
	5 ul 1u Tag: 1mU Tne	
	95 ul H <sub>2</sub> O	
2 (1:1 TT) 1.4 mM Mg <sup>2+</sup>	30 ul 5x A ✓	
	20 ul 5x B ✓	
	5 ul 1u Tag: 1mU Tne	
	95 ul H <sub>2</sub> O	
2 (1:1 TT) 1.5 mM Mg <sup>2+</sup>	25 ul 5x A ✓	
	25 ul 5x B	
	"	
2 (1:1 TT) 1.6 mM Mg <sup>2+</sup>	20 ul 5x A	
	30 ul 5x B	
	"	
2 (1:1 TT) 1.6 mM Mg <sup>2+</sup>	20 ul 5x A	
	30 ul 5x B	
	"	

Take each of these mixes again with the 1:10 TT mix and a mix 2 (1:2 TT) 1.6 mM Mg<sup>2+</sup>

Used & Understood by me, Barbara Polansky	Date 8/7/95	Inv nted by <i>[Signature]</i>	Date 8/2/95
R cord d by <i>[Signature]</i>			

**From Page No.\_\_\_\_**

[illegible]

Time	Temp	Rate
0.15	94°C	30 sec
35 cycles	94°C	30 sec
	60°C	30 sec
	68°C	10 min
	4°C	

Step 1 Full stepwise containing 100mM EDTA (p.79)  
200mM in 0.7% agarose gel, 100V 6'30" - 7<sup>00</sup>

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**Inv nted by**

**Date**

**Record of by**

8/2/95

**To Page** **M**

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE

32P end label Pri 2 --- Pri 2s  
for test of decreasing primer length

From Page No. \_\_\_\_\_

mix for 14 labeling rxns: 28  $\mu$ l  $^32$ P ATP (3.33  $\mu$ M) ref 8/4/95 (6.66  $\mu$ l per rxn)  
 28  $\mu$ l 5x Kinase buffer  
 4.2  $\mu$ l PAK - new lot  
 56.56  $\mu$ l  $H_2O$   
 140  $\mu$ l

rxns = 1. 66  $\mu$ l of each oligo - Fidehpri 2, 3, 4, 6, 8, 10, 12, 14, 16, 18  
 8.34  $\mu$ l mix  $\leftarrow$  10  $\mu$ M stocks (16.6 pmol primer total)  
 100  $\mu$ l in 9600 tubes  
 37 $^{\circ}$ , 30' in Lab 14 9600  
 55 $^{\circ}$ , 5' "  
 cool to 4 $^{\circ}$ C before opening tubes

add 2  $\mu$ l 10  $\mu$ M FideI temp dT - 20 pmol total  
 80 $^{\circ}$ C, 5'  
 cool to Room temp, 15 min 12 9600

add 64.7  $\mu$ l 10  $\mu$ M Tris pH 8.0  
 stored at -20 $^{\circ}$ C overnight

use 42/1000 reaction for 10  $\mu$ M primer

template = 20 pmol = 1.2  
 pri 16.6 pmole

To Page N

Witness d &amp; Und rst d by m ,

D. S. Polansky

Dat

8/7/95

Inv nt d by

R cord d by

Paula L. Smith

Dat

8-2-95

TNE

39 No. \_\_\_\_\_

lig

Q19 / SmaI / SphI .003 pmol/.1  
 b H3 / Gilled in / SphI .015 pmol/.1  
 x ligase buffer  
 H<sub>2</sub>O  
 Ligase (10)

2

1.5

1

4

125

1

200.1

RT - 30 min.

Jason returned 2.1 of the lig with 100.1 DH10B cc.  
 std xform. Plated 10% + 90% on yet amp plates. 37°C ON

#2      10%      90%  
          18      ~150

picked 8 colonies into 3 ml of CG + ampicillin. 37°C - ON.

is mp as usual. Dissolved in 50 µl TE.

mp      3  
 DRRG    2  
 H<sub>2</sub>O      13  
 BpHTE   1  
 E. coli   1  
          10

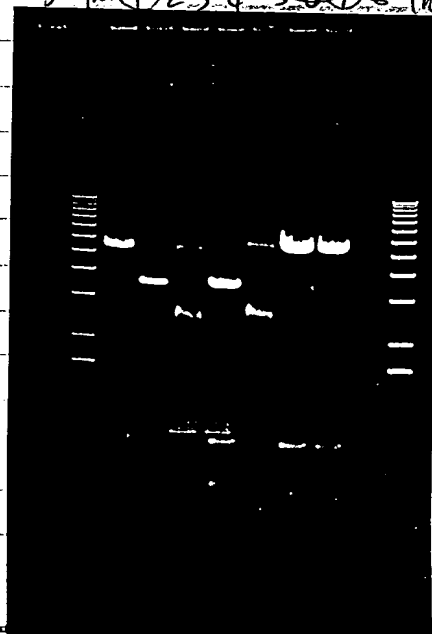
37°C - 1 hr.

Applied to a  
 0.8% agarose  
 gel. Gel  
 run at 100V

4575  
 2000  
 6575

sub PUCTNE 35 PY not into  
 SmaI / SphI site of pTTO19  
 clones cut E SphI / EcoRI  
 5 kb 1 2 3 4 5 6 7 8 9 10

ANY 8/1/95



To Page No. \_\_\_\_\_

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Date

Invent d by

Dat

Lisha Xu

8/3/95

R corded by

CONY Longo

8/13/95

Test extension of short primers  
 $\Delta$  [tag],  $\Delta$  length of primer

From Page No. \_\_\_\_\_

<sup>32</sup>P fid pri: 6 - fide Temp

" 8

" 10

" 12

" 14

" 16

(P140, 250nm primer)

Mix A

46  $\mu$ lTag  $\mu$ l

set buffer 5B

.0016

.007

.04

.2

1

5

2

2

2

2

2

2

2

2

2

2

2

2

2

2

2

2

2

50  $\mu$ l

70°C 20 min 22 min

stop with 25  $\mu$ l cycle seq stop solutionload 3  $\mu$ l on 25% PAGE

(same as P155, 7)

2000V (get ~11mA) for 3 hr

.0032 units (tab 41) 21 pmol ut in 20 min  
(based on units a map efficiency)

input primer is 1 pmol 42 mer

= ~20 pmol ut incorp for 20 nt ssDNA seq

T Page 1

With ss d &amp; Und rstood by m ,

Deborah Solano

Dat 8/7/95

Inv nt d by

Recorded by Pauline Pank

Dat

8-3-95



Age N \_\_\_\_

22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

10 nM primer Cf  
(1 pmol primer total / 50 μl Rx)

---

A 10x10 grid with the number 2 written in the following cells (row, column): (1, 3), (1, 7), (2, 4), (2, 8), (3, 5), (3, 9), (4, 1), (4, 6), (4, 10), (5, 2), (5, 7), (6, 4), (6, 9), (7, 1), (7, 6), (7, 10), (8, 3), (8, 8), (9, 5), (9, 9).

(for 40 Rxns)

mutations:

Jul		
2010	1	2

[illegible]

0.0421

00847

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
---	---	---	---	---	---	---	---	---	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	-----

Mix A

10X PCR buffer  
50 mM MgCl<sub>2</sub>  
10 mM dNTPs

1.540 ml	✓
----------	---

200					✓
-----	--	--	--	--	---

60					✓
----	--	--	--	--	---

40						✓
----	--	--	--	--	--	---

1.240 ml

me 46  $\mu\text{M}$  / K<sub>1/2</sub>

$C_f = 1.5 \text{ mM MgCl}_2$

200  $\mu$ M dNTP of  
in Rxn.

**To Page No.\_\_\_\_\_**

**ed & Understood by me,**

**Date**

Invented by

Date \_\_\_\_\_

Tracy Polay

$$8 \overline{) 7195}$$

**Recorded by**

83-95

**PAGE 144 OF NOTEBOOK WAS BLANK**

Result:

1. primer length 10 and longer and extended by Tag.
2. Will test Tne vs Tag next to see if Tne does better than Tag

Used &amp; Understood by me,

Polamp

Date

8

14/15

Invented by

Recorded by

Date

7-3-95

To Page No. \_\_\_\_\_

From Page No.

32P fid pri 6 • fidel Temp

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

2 —————→

2 —————→

8

10

12

14

16

(P140) 250 nM primer) \*preheated to 70°C w/mix A\*

The diluted in SB #122/95  
P.L.

0.0 156 u/l

2

2

2

0.0 625 u/l

2

2

0.25 u/l

2

2

1 u/l

2

2

Tag 5/31/95 u/l

0.0 156 u/l

2

2

0.0 625 u/l

2

2

0.25 u/l

2

2

1 u/l

2

2

Mix A (P143)

46 u/l

Vp = 50 u/l

20 min 70°C stop with 25 u/l cycle seq  
stop solution

heat to 90°C, 5 min before loading

25 u/l PATE run as P142 and 155, 7, run 3 hr 2000 V

mix A p. 143 scaled up 1.5X = 2.31 mL H<sub>2</sub>O

300 u/l 10X PCR buffer (from Kala)

90 u/l 50 mM MgCl<sub>2</sub> (made from 1M 2/1 sto)

60 u/l 10 mM dNTPS

2760 u/l

50 u/l 1M H<sub>2</sub>O  
950 u/l H<sub>2</sub>O

T Pag N

Withn ssed &amp; Und rst od by m ,

Dat

Inv nted by

Dat

Deanna Polans

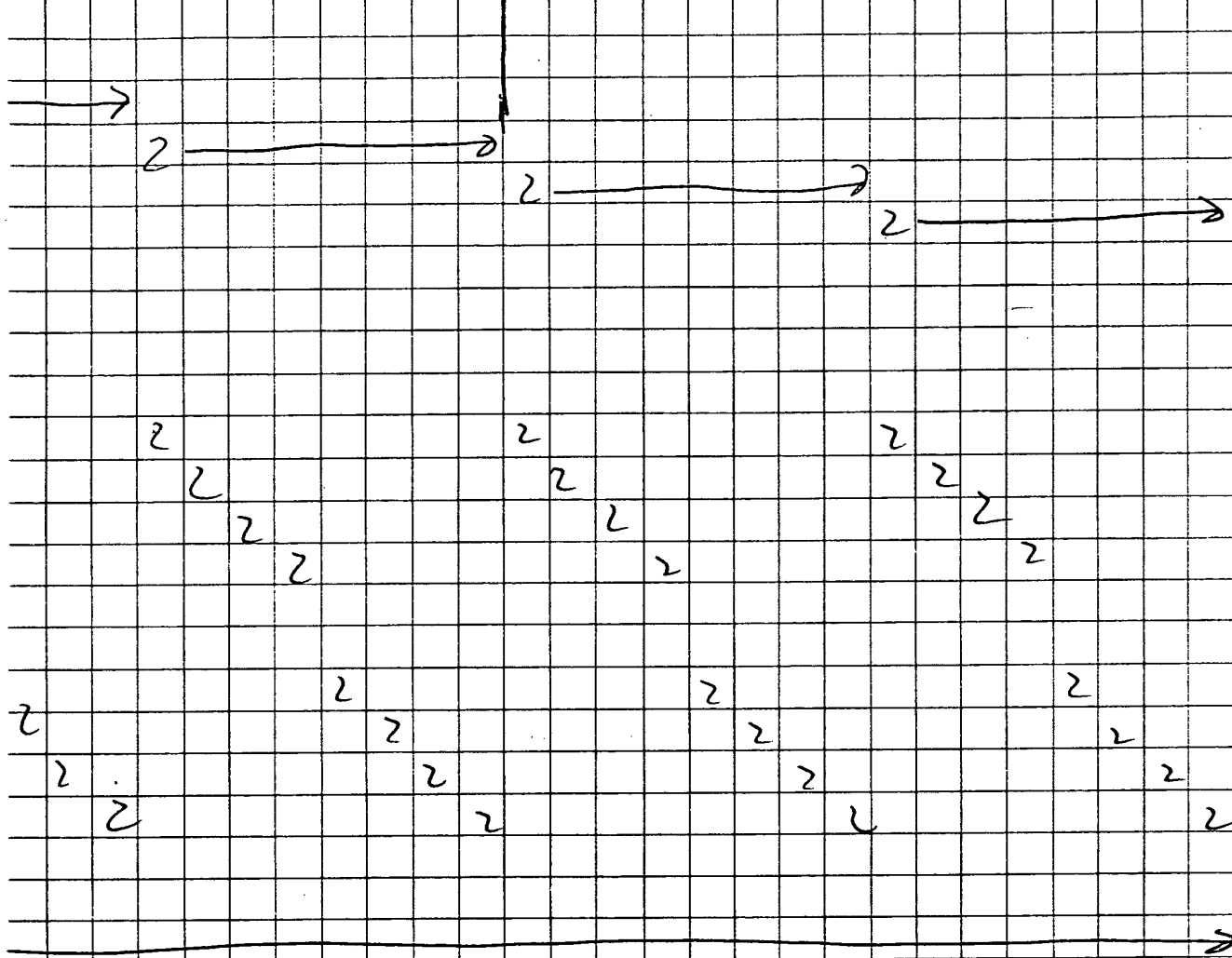
8/7/95

R corded by

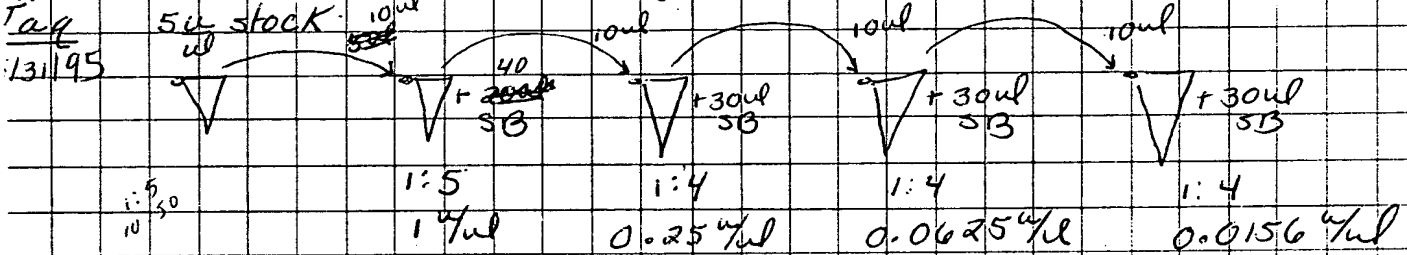
Pawel Pomb

8-4-95

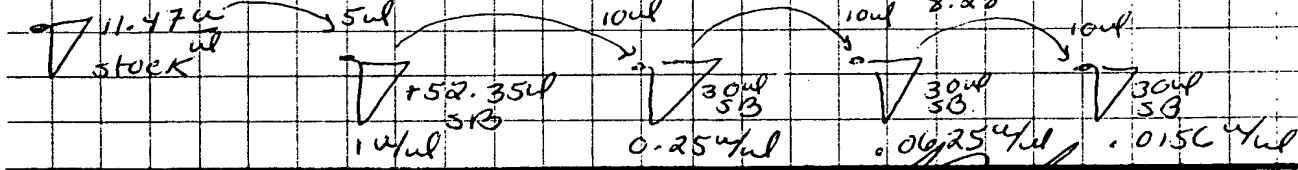
22/23/24/25/26/27/28/29/30/31/32/33/34/35/36/37/38/39/40/41/42/43/44/45/46/47/48



same dilutions: in Tag storage buffer - 12/7/94



The 1st normalize to Tag units p. 127 19 x 5 = 11.47u/l (Tag was thought to be 5u/l)



To Page No. \_\_\_\_\_

Used & Understood by me,

Date

Initiated by

Date

*Michael Polunin*

8/7/95

Recorded by *Paulyn*

*P-4-SJT*

8/5/95

8/5/95

Invented by	<i>Rex Robt</i>
Recorded by	<i>Carolyn Lomb</i>

**From Page No.\_\_\_\_**

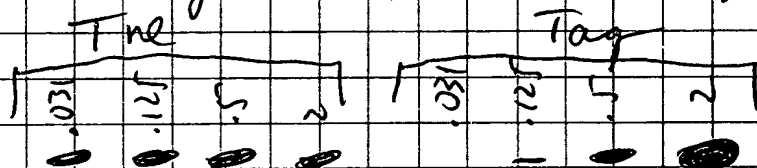
## Results

Data on P 151 152

for primer length 12, 14, 16 the first appearance of run off happens at  $4 \times 16 \times$  less 16x  
units than tag units (based units based on  
tag unit assay P. 127)

at the highest [pol], Tue and Taz have ~ equal extension for  $12^{14}$  nt primers and Taz has greater yield for 16 nt primer at highest level

ii. In 16mer primer, T<sub>2</sub> again shows first appearance of ~~amplification~~ runs of but T<sub>2</sub> has greater yield at 12 units/enzyme  $\frac{80}{100}$



less Tne is needed to extend a primer.  
Maybe Tne is more processive  
That is consistent with Tag giving so  
extensive (equal or better than Tne) at high  
and ~~constant~~ could explain how Equal unit  
of Tag, Tne for DNase I treated gaps  
DNA is act differently for primer exten.

T Pag M

Witness d & Und rsto d by ~~me~~.

## Dat

Invited by

**Dat**

Recorded by

ag N — see p. 140

nd-label Fid 16: 4  $\mu$ l  $^{32}$ P $\gamma$ ATP ref 8/4/95  
48.08  $\mu$ l H<sub>2</sub>O4  $\mu$ l 5x Kinase buffer3.32  $\mu$ l 10  $\mu$ M Fid 16 oligo (33.2  $\mu$ M, 4.46 pmol/run)4  $\mu$ l  $^{32}$ P $\gamma$ ATP ref 8/4/95 (3.33  $\mu$ M, 4.46 pmol/run)0.6  $\mu$ l PNK20  $\mu$ l in 9600 tube

37°C, 30 min ✓

55°C, 5 min ✓

cool to ~4°C ✓

+ 4  $\mu$ l Fidel Temp, 10  $\mu$ M 42mer (40 pmol)

80°C, 5 min

cool for 15 min to RT in PCR machine

+ 129.4  $\mu$ l 10 mM Tris pH 8.0

store at -20°C

To Page No. \_\_\_\_\_

sed &amp; Understood by me,

J Polay

Date

8/14/95

Invented by

Recorded by

Cawlyn Comb

Date

8/7/95



**PAGES 150-151 OF NOTEBOOK WERE BLANK**

32p 42 mer for 3' exo assay  
of 3' exo (-) Klenow fragment

ag N. \_\_\_\_\_

End Template 10 $\mu$ M (42 mer)	5.8 $\mu$ l	✓	5 $\mu$ mol total
3-P-ATP	7	✓	
5x Klenow buffer	7	✓	
PNK 1 $\mu$ l	2	✓	
H <sub>2</sub> O	13.2 $\mu$ l	✓	
	35		

37°C, 30'

70°C, 5'

1 M KPO<sub>4</sub> pH 7.6

1 M Kmonobasic 1.3 ml

1 M K dibasic 8.7 ml

VP = 10 ml

To Page No. \_\_\_\_\_

Read & Understood by me,

Researcher a Golamp

Date

8/14/95

Invented by

Recorded by

Date

8-8-95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Primer extension: Fid 16 primertime course with  
4 [eng], Tag vs Tne

From Page No. \_\_\_\_\_

purpose: In the last primer extension experiment, ~4x less Tne than Tag was able to extend the Fid 16 primer to a full length 42mer product. p. 150. The <sup>highest</sup> level of Tag (2u), that was tested, made even more full-length product than the highest level of Tne tested (2u). ~~Today's expt.~~ The purpose of today's expt. is to confirm the results of p. 150 and to determine whether Tne & Tag have different affinities for DNA/primer binding or if they are the same but Tne is better at extending after a stop. <sup>the time course & no eng run will reveal if the enzyme stops</sup> Sequencing rxns and the Fid 16 primer without enzyme will also be run on the 25% gel.

materials:PCR/Mg<sup>2+</sup> mix to mix with enzyme prior to beginning rxns:

25 $\mu$ l	10x PCR mix	✓	conc
7.5 $\mu$ l	50mM MgCl <sub>2</sub>	✓	1.25x
147.5 $\mu$ l	H <sub>2</sub> O	✓	1.25x
<hr/> 200 $\mu$ l			

2 use 16  $\mu$ l ✓+ 4  $\mu$ l of eng. dilution in 5B ✓20  $\mu$ l - 1x PCR buffer = 20mM Tris 8.4, 50mM  
tubes 1-8 1x MgCl<sub>2</sub> CF = 1.5mM

mix A for 10 rxns:

80 $\mu$ l	10x PCR buffer	✓
63 $\mu$ l	H <sub>2</sub> O	✓
24 $\mu$ l	50mM MgCl <sub>2</sub>	✓
20 $\mu$ l	10mM dNTPs	✓
40 $\mu$ l	<sup>32</sup> P Fid 16 annealed to Fid 1 Temp p. 14	
<hr/> 200 $\mu$ l		

Keep at 70°C

enzyme dilutions in Tag storage buffer: same as on p. 147  
same preps of Tag & Tne5  $\mu$ l STOP soln. from cycle sequencing kit in 9600 tubes 1-40

T Page N

Witnessed &amp; Understood by m ,

Date

Investigated by

Date

D. Polansky

8/

11/1/95

Recorded by

Paula L. Paul

8/8/95

'ag N .\_\_

#	1	2	3	4	5	6	7	8
tubes	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40

2/95 1 2 5 3 30  
0 3 4 5 all prewarmed to 70°C

1 The mix	20
-----------	----

25 PCR  
fer + mg<sup>2+</sup>

7 Tag mix						20
-----------	--	--	--	--	--	----

[illegible][illegible][illegible]

'5 <sup>u</sup> / <sub>u</sub> Tag mix	20
--	----

[illegible][illegible]

4 rxns w/ 30  $\mu$ l of mix A prewarmed to 70°C, titrate w/ p200

move 10ul of rxn. to a 9600 tube containing 5ul STOP at  
1min, 2min, 5min, 10min, 20min - on ice

at to  $80^{\circ}\text{C}$ , 5 min before loading  $1.5 \mu\text{l}$  on 25% gel

ter of loading: on 25% urea gel

left ATGC noenz ~~31-40~~ ↑ 21-30 ↑ 11-20 ↑ 1-10

↓	16 $\mu$ l	1.25X PCR buffer
	4 $\mu$ l	storage buffer
	+ 80 $\mu$ l	mix A

no  
en3  
Fid 16

no en2  
F. d 16

no enzy  
F1d16

diluted  
10<sup>4</sup>X  
to 1x10<sup>-3</sup> ul

↳ removed 10  $\mu$ l +  
5  $\mu$ l stop - from cycle sequencing kit

ded on 8% gel in same order loaded 1.5ul

To Page No. 104

**ed & Understood by me.**

Date \_\_\_\_\_

**Inv nted by**

## Dat

Polans

8

Recorded by

Caroleen Comb

8/8/95

From Page No. \_\_\_\_\_

10 Rows

Tm PCR  
condition

BSA 20mg/L	✓	A 2.5 ul	B 1
1M $\text{KH}_2\text{PO}_4$ pH 7.6	✓	25	5
10X PCR buff	✓		50
50 mM $\text{MgCl}_2$	✓	70	70 mg
50% sterile glycerol	✓	140	1
H <sub>2</sub> O (sterile)	✓	232.5	350
3:42 min	✓	10	10
(fidel Template P61)			
* see P 74-75		480	480
* Beckman Oucherson			

3:42 min	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
48 min																		
48 min																		

Klenow exo-																		
lot CK041 175 u/L																		
dil 25 u/L	2									2								
5 u/L		2								2								
1 u/L			2								2							
Klenow exo(-)																		
lot EJIP41 130 u/L																		
dil 25 u/L			2									2						
5				2									2					
1					2									2				
Klenow (+) exo																		
0.6 u/L						2								2				
0.006							2								2			

dilutions  
with T<sub>1</sub>  
dil buff

start 1:2

3 hr

25 ul cycle  
stop solload 3 ul  
8% PA

Witness d &amp; Und rst od by me,

Polamp

Dat

8/14/95

Inv nted by

R c rded by

Dat

8-9-95

To Pag N

From: Hartman, Chris  
 T : Lasken, Roger  
 C : Rashtchian, Ayoub  
 Subject: Exo minus Klenow  
 Date: Monday, August 10, 1995 3:40PM  
 Pri rity: High

roj ct No. \_\_\_\_\_  
 Torg No. \_\_\_\_\_  
 PERL  
 buffer

P63, 10  
 63

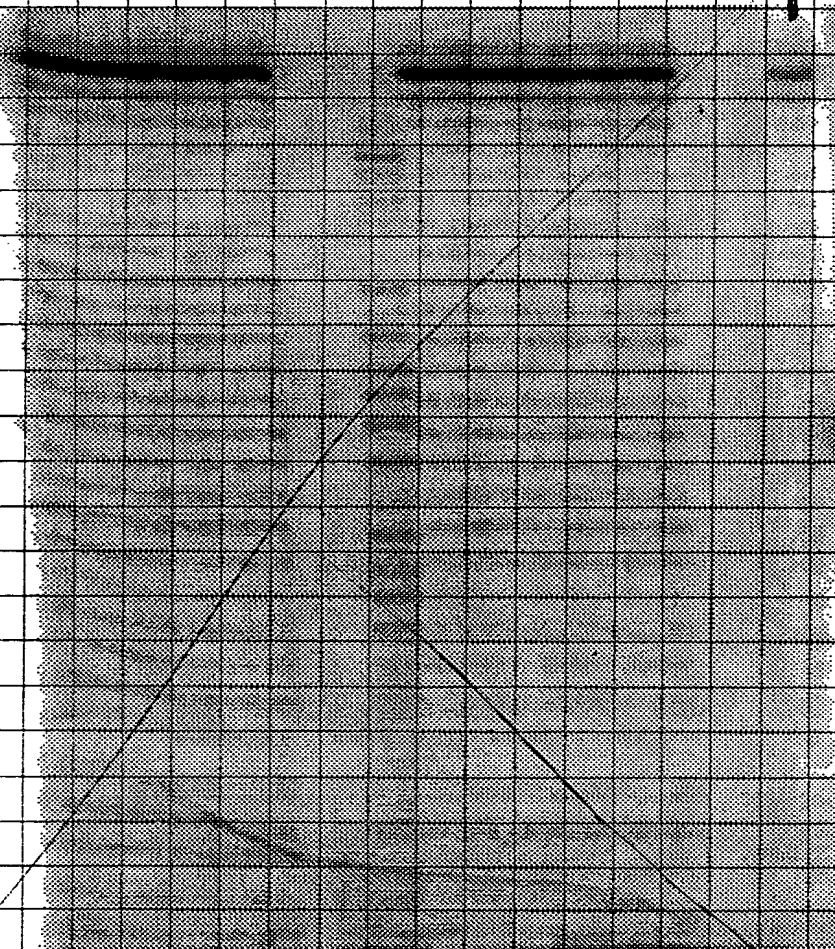
Pecton  
 Dickinson  
 Conditions

Roger, The unit values for the bulk exo minus klenow are as follows.

Lot No. U/ul  
 CK041 75  
 EJP41 130

Klenow Exo(+)						Klenow Exo(+)						Enzyme	
CK041			EJP41			CK041			EJP41				
50	10	2	50	10	2	50	10	2	50	10	2	1.2	0.12

P42 mer



To Page No. \_\_\_\_\_

s d & Understood by m ,

*Polay*

Date

8/14/95

Invent d by

*[Signature]*  
Recorded by

Date

8-14-95

From Pag No. \_\_\_\_\_

33 correct (p138, 9)  
20  $\mu$ M4.36  
✓ 2.18  $\mu$ l87  
~~43.0~~ p  
hr

M13 mp19 s DNA (+)

✓ 200  $\mu$ l21.8 pmol  
total

1M Tris pH 7.5

✓ 10.6  $\mu$ l✓ 212.78  $\mu$ l50 mM  
Tris(00 pmol  
long)0.1025 pmol circle /  $\mu$ l  $\Rightarrow$  743 pmol nt /  $\mu$ l  
use 2  $\mu$ l / 50  $\mu$ l Rxn for 1.5 nmol nt / Rxn

Mix A

(33.4 Rxns)

Taps MgCl<sub>2</sub> KCl  
(of p120, 9)(add 15  $\mu$ l  
next time)103  $\mu$ l ✓320  $\mu$ l ATP 10mCi/ml 300  $\mu$ l pmol

3 ✓

+ ATP 10mM

33.4 ✓

(200  $\mu$ M)

+ CTP 10mM

33.4 ✓

+ GTP 10mM

33.4 ✓

+ Tag 5  $\mu$ l66.8  $\mu$ l ✓

33. mp19

66.8  $\mu$ l ✓743 pmol nt /  $\mu$ lH<sub>2</sub>O

1.163 ml ✓

1.503 ml

10  $\mu$ l / 50  
1.5 nmol ntuse 45  $\mu$ l / 50  $\mu$ l Rxn

T Pag

With ss d &amp; Understo d by m ,

S. Polansky

Dat

8/14/95

Invented by

Rec rd d by

Dat

F. S. J.

**Recorded by**





Processivity of Tag, Tne, and Ultima

Tag N \_\_\_\_\_

extension of 33-mer correct primer annealed to m13mp19 ssDNA  
 serial eny dilutions, 2 min extension and 10 min endpt extension  
 2 units - 0.0078 units, in 50ul rxns, reactions started w/ 2ul eny.

1/9/95

action cocktail for 35 rxns = 175ul 10x PCR buffer  
 1347.5ul H<sub>2</sub>O

ote: 42 <sup>32</sup>P primer: 1 m13 circle 52.5ul 50mM MgCl<sub>2</sub>

\* mistake, see 8/11/95 35ul 10mM dNTPS

were 2ul of the labeled 70ul <sup>32</sup>P-33mer correct annealed

\* annealed primer was  
 diluted w/ 70ul m13

1680ul

to m13mp19 - the Kinase  
 rxn was done as on

p. 12 NB10, then 46ul

of m13 ssDNA added

0.26ug/ul m13 stock

" 32 P33 correct m13 "

this was 42 pri/circle - 8/11/95  
 where more m13 added to get 1 pri/circle

zyme dilutions in Tag SB

Tag, 5/31/95, 5ul/ul

5ul/ul → 4ul/ul → 16ul SB

1:5

1ul/ul

10ul/ul → 10ul/ul → 10ul SB

1:2

0.5ul/ul

0.25ul/ul

2 fold dilutions

0.125

0.0625

0.0313

0.0156

0.0078, 0.0039ul

mutarray 7/30

Tne 7/31/95

11.47 ul/ul

5ul

11.47 ul/ul

+ 52.35ul SB

1:11.47

= 8.1ul/ul

- this value is normalized to Tag p. 147

RL's Tne of 7-22-95

then, serial dilutions made in the  
 same way as for Tag

Ultima

6ul/ul

7ul/ul

20ul SB

1ul/ul

Lot 0643 12/31/95, Perkin Elmer

same dilutions as for Tag & Tne, but  
 Ultima units are not normalized to  
 Tag units

To Page No. \_\_\_\_\_

Read & Understood by me,

DPolamp

Date

8/14/95

Invent d by

Recorded by

Carolyn Conn

Dat

8/9/95

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_ TITLE \_\_\_\_\_

From Page N. \_\_\_\_\_

- ~~2.5~~ 48  $\mu$ l mix in a 9600 PCR tube, preheated to 70°C
- reactions were started by adding 2  $\mu$ l of enzy w/ P<sub>2</sub> and triterating w/ P<sub>200</sub>
- after 2 min at 70°C, in 9600, rxns were stopped w/ 25  $\mu$ l Cycle sequencing Stop solution and kept at -20°C overnight prior to loading on 8% gel

Tubes	1 — 10	11 — 20	21 — 30
	Taq	Tne	Tma
	0.0078 → 20 units	0.0078 → 20 units	0.0078 → 20 units

The 20 units rxns were incubated for <sup>10</sup>min; while all the other rxns were incubated for 2 min

T Pag N.

Withn ssed &amp; Und rst d by m ,

D. Polansky

Dat

8/14/95

Inv nt d by

R corded by

D. Polansky

Dat

8/9/95

Project No. \_\_\_\_\_  
 Book No. \_\_\_\_\_

TITLE Extension of 16-mer by Tag + Inc  
with  $\Delta$  MgCl<sub>2</sub> +  $\Delta$  KCl

158

From Page No. \_\_\_\_\_

2

general overview of conditions tested:

fix MgCl <sub>2</sub>	<u><math>\Delta</math> KCl (mm)</u>	} each condition tested w/ 0.0312u Tag + Inc 2.0u Tag + Inc in 50ul rxns at = for 20min
1.05 mm	0 25 50 85	
1.5 mm	0 25 50 85	
fix KCl	<u><math>\Delta</math> MgCl (mm)</u>	} = 44 rxns
50 mm	1, 1.2, 1.5	

for 1, 50ul rxn: 1ul 1M Tris <sup>8.5</sup> ~~8.4~~ CF=20mm \* note that real pCK  
 41.08ul H<sub>2</sub>O buffer is pH 8.4  
 1.42ul 3M KCl for 85mm CF  
 1.5ul 50mm MgCl<sub>2</sub> for 1.5mm CF  
 1ul 10mm dNTP CF=200uM  
 \* 2ul <sup>32</sup>P 16mer on Fide Temp CF=10mm  
 2ul enz to start rxn  
 50ul

\* End-label 16mer as on p. 149  
 mix A for 50 rxns: 50ul 1M Tris, pH 8.5 ✓ 22.5 ✓  
 1875ul H<sub>2</sub>O ✓ 843.75 ✓  
 50ul 10mm dNTP ✓ 210ul H<sub>2</sub>O <sup>22.5</sup> ✓  
 100ul <sup>32</sup>P 16mer annealed to Fide Temp (✓)  
 2075ul

T Pag 1

Witn ss d & Underst d by m ,

Dat

Inv nted by

Dat

DD Polamp

5/14/95

R cord d by  
 Darolm Pomh

8/10/95

ig N \_\_\_\_\_

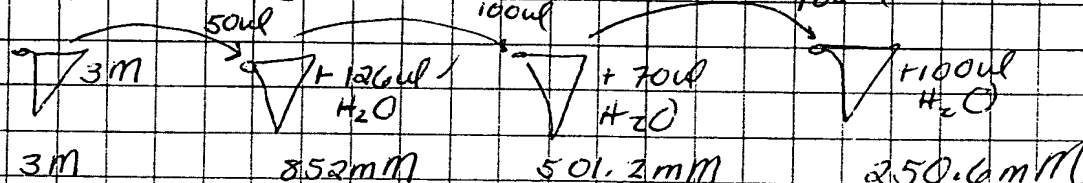
the expt w/ varied KCl

B w/ 10.05 mM MgCl<sub>2</sub>

7 rxns

705.5  $\mu$ l A17.85  $\mu$ l 50 mM MgCl<sub>2</sub> ✓✓7.65  $\mu$ l H<sub>2</sub>O ✓✓731  $\mu$ l2 rxns 180.6  $\mu$ l B C<sub>f</sub> = 0 mM KCl  
+ 21  $\mu$ l H<sub>2</sub>O → 48  $\mu$ l / rxn  
4 tubes 2 rxns = 4180.6  $\mu$ l B+ 21  $\mu$ l 250.6 mM KCl C<sub>f</sub> = 25 mM KCl / rxn180.6  $\mu$ l B+ 21  $\mu$ l 501.2 mM KCl C<sub>f</sub> = 50 mM KCl180.6  $\mu$ l B+ 21  $\mu$ l 852 mM KCl C<sub>f</sub> = 85 mMC w/ 10.5 mM MgCl<sub>2</sub>705.5  $\mu$ l A25.5  $\mu$ l 50 mM MgCl<sub>2</sub> ✓731  $\mu$ lsame but use  
180.6  $\mu$ l C for each

Serial dilution of 3M KCl stock: ✓



To Page No. \_\_\_\_\_

d &amp; Understood by m ,

Date

8

Invented by

Date

Recorded by

8/10/95

Polcup

14/95

C. J. J. J. J.

From Page No. \_\_\_\_\_

For expt w/ varied  $[Mg^{2+}]$ :

for 14 rxns

D

= 581  $\mu$ l A11.62  $\mu$ l 3M KCl ✓9.38  $\mu$ l  $H_2O$  ✓

C = 50 mM KCl / rxn

for 4.2 rxns

602  $\mu$ l180.6  $\mu$ l D+ 21  $\mu$ l 10 mM  $Mg^{2+}$  /use 48  $\mu$ l / rxnC = 1 mM  $Mg^{2+}$  / rxn180.6  $\mu$ l D+ 21  $\mu$ l 12 mM  $Mg$  ✓C = 1.2 mM  $Mg$  / rxn180.6  $\mu$ l D+ 21  $\mu$ l 15 mM  $Mg^{2+}$ C = 1.5 mM  $Mg$ dil of 50 mM  $MgCl$  stock:50  $\mu$ l  
150 mM50  $\mu$ l  
+ 116.5  $\mu$ l  
 $H_2O$   
15 mM100  $\mu$ l  
+ 25  $\mu$ l  
 $H_2O$   
12 mM100  $\mu$ l  
+ 20  $\mu$ l  
 $H_2O$   
10 mMEnzyme dilutions in Taq storage buffer<sup>(SB)</sup>:Taq, 5/31/95 stock 5  $\mu$ l (not real units)5  $\mu$ l  
5  $\mu$ l40  $\mu$ l  
SB  
14  $\mu$ l10  $\mu$ l  
631  $\mu$ l  
SB  
0.0156  $\mu$ lTne, 7/22/95 stock = 11.47  $\mu$ l, normalized to Taq p. 127 + 14711.47  $\mu$ l52.35  $\mu$ l SB  
14  $\mu$ l10  $\mu$ l  
631  $\mu$ l  
SB  
0.0156  $\mu$ l

With ss d &amp; Und rst d by m ,

Dat

8/14/95

Inv nt d by

R cord d by

D. J. P. P. P.

Dat

8/10/95

T Pag 1



Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

From Page No. \_\_\_\_\_

25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

.5mg OKel 48

.25mg 48

.50mg 48

.35mg 48

.50mg .1mg

.1.2mg

.1.5mg

.0156 Tne

.0156 Tag

1 Tne

2 2 2 2

1 Tag

2 2 2 2

200V, 40mA 12 PM -  
12:50 PM

T Pag N

With ssed &amp; Und rst d by m ,

Date

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D. Polansky

8/14/95

Record d by

D. Polansky

8/10/95



Redid processivity of P155  
expect expand slower [pol7ran]

From Page No. \_\_\_\_\_

and correct error in pri / ~~per~~  
from 42 to (here) 30

32P 32 correct • m p19  
P155

2  $\mu$ l

m p19 0.26  $\mu$ g /  $\mu$ l

70  
70  $\mu$ l

2.6 pmol circles  
total  
now travel  
pri / circle = 1

10 x PCR buffer  
H<sub>2</sub>O  
50 mM MgCl<sub>2</sub>  
10 mM dNTP

mix A  
70  $\mu$ l  
175  $\mu$ l  
1347.5  $\mu$ l  
52.5  $\mu$ l  
35

VF = 1670  $\mu$ l

33. m p19

~ 0.22 pmol circles  
per 50  $\mu$ l rxn

(preheat to 70°C  
47  $\mu$ l mix A + 2  $\mu$ l of pol  
to start  $\rightarrow$  kill with 25  $\mu$ l  
cycle seq stop sol

same (enz) as p. 155 and 5 more 2 fold dilutions

Tube 15 ✓  
20 units / 50  $\mu$ l rxn

Tube 14  
2 ✓

(units / rxn)

Tag = 1-15

0.156 0.0078 0.0038 0.00194 0.000

Tne = 16-29

0.000484 0.000242 = tube 1

T Page N

With ss d & Understood by m ,

Dat

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8/11/95

Polamp

From: Hartman, Chris  
 To: Lasken, Roger  
 Subject: Rashtchian, Ayoub  
 Date: Exo minus Klenow  
 Priority: Monday, August 07, 1995 3:40PM  
 High

Project No. — Exhibit L-157  
 Book No. — Appl. No. 09/558,421

P63, 10 63

Section  
 Dickinson  
 Conditions

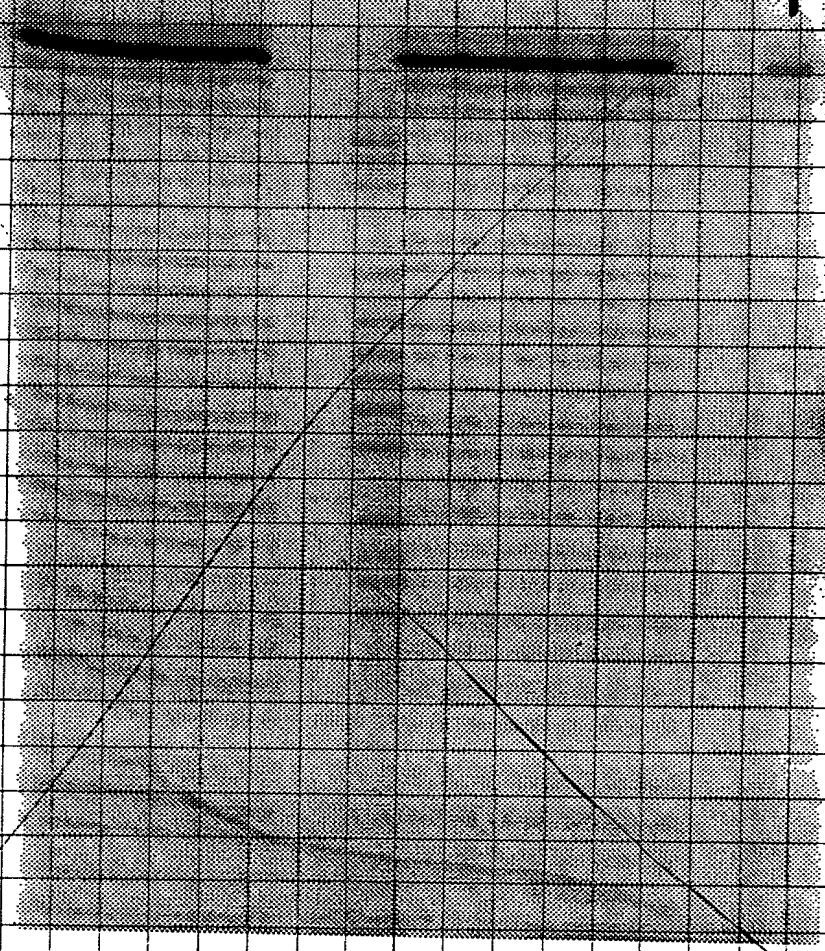
Tag  
 PCR  
 buffer

Roger, The unit values for the bulk exo minus klenow are as follows:

Lot No. U/ul  
 CK041 75  
 EJP41 130

Klenow Exo(+)						Klenow Exo(+)					
CK041			EJP41			CK041			EJP41		
50	10	2	50	10	2	50	10	2	50	10	2
1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2

P42 mer —



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 Polamp

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Date  
 8-14-95

To Page No. —

Test supernix for (JATP)  
[JATP] [JCTP]

From Page No. \_\_\_\_\_

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

- 67 ① (-JATP)  
② (-JCTP)  
③ (-JCTP)

4 JATPs

2.5  $\mu$ m  
5  $\mu$ m  
10  $\mu$ m  
20  $\mu$ m  
40  $\mu$ m  
60  $\mu$ m

\* Mix B #11

2.5  
5  
10  
20  
40  
60

H<sub>2</sub>O5  
30  $\mu$ l

- assemble on ice
- put in 2600 4°C

sample to 70°C  $\Rightarrow$  45"  
sample to 4°C

\* for B  
its 220  $\mu$ m  
(at 1.1x)  
30  $\mu$ l B  
30  $\mu$ l H<sub>2</sub>O  
4 = 110  $\mu$ l

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JOP oiaup

Dat

8/21/95

Invent d by

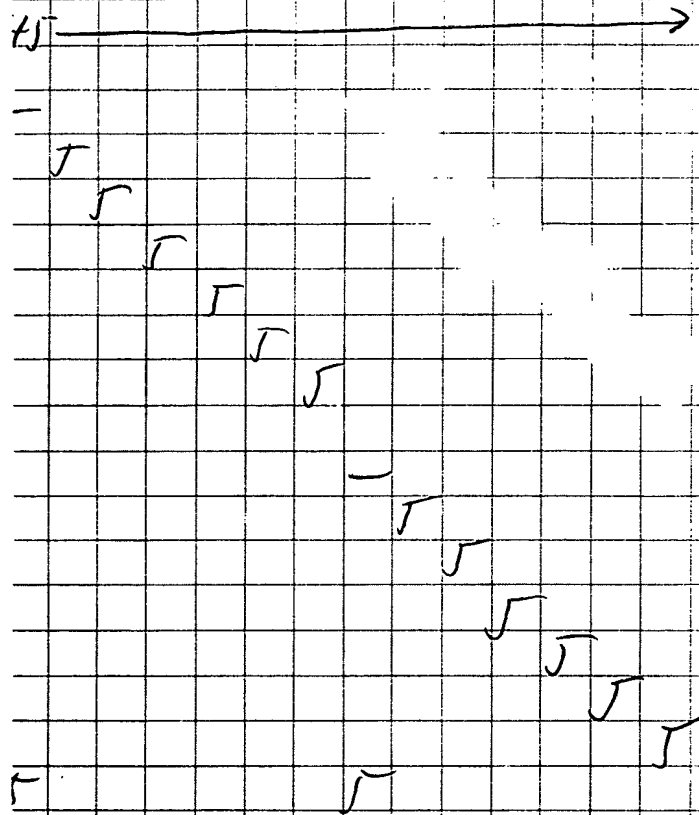
R c rded by

Dat

8-15-95

T Pag 1

30 31 32 33 34 35 36 37 38 39 40 41 42



43 is mix (1) ice only  
ice no 70°C before EOTF

44 is mix (3) ice only

45 2<sup>nd</sup> # Rxn # 15 }  
46 2<sup>nd</sup> # Rxn # 29 } no TCA

To Page No. \_\_\_\_\_

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

Route P 65

From Page N . . .

pmsol

1	103.00	3.5
2	264.00	148
3	275.00	15.5
4	638.00	41
5	1025.00	68
6	1248.00	74
7	2048.00	145
8	56.00	2.2
9	204.00	11
10	297.00	17
11	619.00	40
12	1011.00	67
13	1430.00	96
14	1879.00	128
15	94.00	2.9
16	488.00	30
17	653.00	42
18	1332.00	89
19	2200.00	150
20	3902.00	273
21	5706.00	385
22	83.00	2.1
23	457.00	28
24	643.00	41
25	1289.00	86
26	2218.00	151
27	4406.00	304
28	4082.00	282
29	53.00	
30	57231.00	

42.9 cpm pmsol

1	768.00	8.1
2	854.00	32
3	926.00	53
4	928.00	53
5	979.00	68
6	1443.00	198
7	1721.00	277
8	818.00	22
9	763.00	6.8
10	971.00	65
11	931.00	54
12	1155.00	117
13	1230.00	138
14	1473.00	207
15	16.00	
16	739.00	
17	14195.00	

not very high ? H<sub>2</sub>ATP BK6D

(14195 cpm)

(50 pLR<sub>20</sub> + 10 pLR<sub>EDTA</sub>)

(2 x spotted)

(40000 pmsol<sub>20</sub> / 7.5m)= 10.64 cH  
To Pag

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE Determination of how to kill DNA

166

From Page No. \_\_\_\_\_

DNase I will be used to treat the enz prep. If DNA contamination is the cause <sup>(primer dimers)</sup> of the smear, then the DNase I treatment may eliminate the smear. 1<sup>st</sup> we'll establish how to kill DNase I, after it has been mixed w/ Tne, so it won't be active during a

1. DNase I rxn will be killed w/ CDTA + heat for 5, 10 + 30 min.  
A second DNase I rxn will be killed ~~only~~ by heat only for 5, 10, 30.  
After the killing treatment, the rxn will be mixed with QX174 RF.  
If the DNase I was killed, the QX174 won't be degraded, even after a 3hr incubation.

materials: QX174, 0.25  $\mu$ g/ $\mu$ l in 0.1mm CDTA from LTI Lot FA370.

DNase I, 1  $\mu$ l in SB = 20mm NaOAc pH 6.5  
5mm CaCl<sub>2</sub>  
50% glycerol

25mm CDTA - 50ul 0.5M CDTA pH 8  
950ul H<sub>2</sub>O

200mm Tris 8.5 (note: the DNase I buffer is 8.4)  
200ul 1M Tris 8.5  
800ul H<sub>2</sub>O

20mm MgCl<sub>2</sub> - 200ul 50mm MgCl<sub>2</sub>  
+ 300ul H<sub>2</sub>O

200mm KCl - 200mm 66.7ul 3M KCl  
+ 933.3ul H<sub>2</sub>O

10X DNase I buffer = 200mm Tris-HCl pH 8.4  
LTI 20mm MgCl<sub>2</sub>  
Lot EK2410 500mm KCl - it was a bit  
bubbly after  
mixing

0.8% TAE agarose gel w/ EtBr

T Page 1

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8/15/95

Page N. \_\_\_\_\_

DNase I rxns: in 9600 PCR tubes

1) 14  $\mu$ l  $H_2O$   
 + 2  $\mu$ l 10x DNase I buffer  
 + 4  $\mu$ l DNase I, 14  $\mu$ l  $C_F = 0.2 \mu$ l  
 20  $\mu$ l  
 Kill with  
 heat + CDTA

(2) 14  $\mu$ l  $H_2O$   
 2  $\mu$ l 10x DNase I buffer  
 4  $\mu$ l DNase I  $C_F = 0.2 \mu$ l  
 20  $\mu$ l - only heat kill this rxn

15' at  $RT (= 23^\circ C)$  → during this time 1  $\mu$ g  
 DNA should be digested

remove 4  $\mu$ l (= 0.8 units DNase I)  
 + 4  $\mu$ l 0.25  $\mu$ g/ $\mu$ l  $\phi$ X174  
 + 1.6  $\mu$ l 10x DNase I buffer  
 + 10.4  $\mu$ l  $H_2O$   
 20  $\mu$ l w/ 1  $\mu$ g

(series to see how long it takes active  
 DNase I to degrade 1  $\mu$ g  $\phi$ X174)

→ immediately remove 4  $\mu$ l + 1  $\mu$ l 10x loading dye w/  
 100 mM CDTA = 0' Kill time  
 0 time incubation w/  $\phi$ X174  
 → 2' ~~later~~ remove 4  $\mu$ l + 1  $\mu$ l LD = 0' Kill time  
 2' w/  $\phi$ X174  
 → 15' ~~later~~ remove 4  $\mu$ l + 1  $\mu$ l LD = 0' Kill time  
 15' w/  $\phi$ X174  
 → 1 hr ~~later~~ remove 4  $\mu$ l + 1  $\mu$ l LD = 0' Kill time  
 1 hr w/  $\phi$ X174  
 → 3 hr remove 4  $\mu$ l + 1  $\mu$ l LD = 0' Kill time  
 3 hr w/  $\phi$ X174.

⑦ Kill the remaining 16  $\mu$ l w/ 1.36  $\mu$ l 25 mM CDTA  $C_F = 2$  mM  
 ①+② heat to  $75^\circ C$  in 9600

→ 5' of heat kill, remove 4  $\mu$ l  
 for rxn ①

+ 4  $\mu$ l  $\phi$ X174  
 + 4  $\mu$ l 200 mM KCl  $C_F = 50$  mM, 16  $\mu$ l  
 + 1.6  $\mu$ l 200 mM Tris 8.5  $C_F = 20$  mM, 16  $\mu$ l  
 + 2.02  $\mu$ l 20 mM  $MgCl_2$   $C_F = 2.02$  mM  
 + 4.4  $\mu$ l  $H_2O$   
 20  $\mu$ l → 3 hr at  $RT$   
 $25^\circ C$

To Page No. \_\_\_\_\_

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J. J. J. J.

Date

8/21/95

Inv. nt d by

Recorded by

Cawlyn Combs

Date

8/15/95



From Page No. \_\_\_\_\_

remove 4ul aliquots and treat in the same way after 15' and 30' Killing with heat and EDTA

for rxn ②

for the DNase I rxn that was killed by only heat, take 5', 15', and 30' killing time points by removing 4ul of rxn to a tube w/

4ul  $\phi$ X174, 0.25  $\mu$ g/ $\mu$ l  
4ul 200mM KCl  
10.6ul 200mM Tris 8.5  
10.6ul 20mM MgCl<sub>2</sub>  
4.8ul H<sub>2</sub>O  
20ul

- 3hr incubation at RT
- + 3ul 10x Loading dye w/ 100% EDTA
- run 23ul on 0.8% gel

gel order

14 wells

1Kb ladder 10ul	1ul $\phi$ X174	5' heat + EDTA	5' heat only	15' heat + EDTA	15' heat only	30' heat + EDTA	30' heat only	OK!! 10ul $\phi$ X174	OK!! 2ul $\phi$ X174	OK!! 15ul w/ $\phi$ X174	OK!! 1hr w/ $\phi$ X174	OK!! 3hr w/ $\phi$ X174	1Kb ladder
--------------------	-----------------	-------------------	-----------------	--------------------	------------------	--------------------	------------------	--------------------------	-------------------------	--------------------------------	-------------------------------	-------------------------------	------------

active DNase I  
incubated w/ 1  $\mu$ g  $\phi$ X174  
varying amounts of time

T Pag N

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Inv nt d by

R cord d by

Paula P. Smith

Dat

8/15/95

with assay for -20°C sample  
from J80les and also 1.1X mix sum  
as P 34, 52, 80 (with assay P 18)

From Page No. \_\_\_\_\_

From Page No. =

(note -20°C #11 array)  
(on P 18)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

-20 #1E 27/1

2 2 2

-20 #4E 27/1

2 2 2

-20 #7E 27/1

2 2 2

1.1X J8-95

2 2 2

1.1X Field Test  
(old on P 34)

2 2 2

r Tag 1/25 dil  
J-30-95

2 2 2 2 2

Tag Rem in  
P120, 8

48 ml

relative  
to Tag

P1225  
(time 0)

cpm

u/ml

u/1

-20 1E

25 5911.00

26 6883.00

27 6801.00

28 5982.00

-20 4E

29 5759.00

30 5205.00

31 6079.00

-20 7E

32 5062.00

33 7422.00

1.1X  
J-8-95

34 4974.00

35 4594.00

36 4752.00

1.1X  
old Field  
Test

37 4389.00

38 4552.00

39 4971.00

40 8930.00

r Tag

41 8601.00

42 8299.00

43 8980.00

GK60

44 8618.00

45 78.00

46 100897.00

2X  
mix

47 102480.00

48 102152.00

6531

.03

.037

81%

see P 53  
wine #11  
of 27/1  
54% incl  
renew  
8% #1E 4  
show bottle

5649

.026

.029

90%

6187

.028

.033

85%

4773

$\frac{4773}{8686} = .04$

.022

$\frac{.022}{.023(P34)} =$

96%

4637

$\frac{4637}{8686} =$

.0213

$\Rightarrow$

93%

8686 and

.04

(by  
definition)

To Page 1

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Date

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Date

J. Polansky

8/21/95

Rec'd d by

8-11-95

From Page No. \_\_\_\_\_

specific activity of A mix =  $\frac{101843 \text{ cpm}}{40,000} \left( \frac{50 \mu\text{l}}{2 \mu\text{l}} \right) = 63.7 \text{ cpm/pmol nt}$ 

$$p\text{mole} = \frac{\text{cpm}}{S.A.} \left( \frac{60}{20} \right) (\text{pmol})$$

SAM	CPM1	
1	3072.00	-147
2	1533.00	-72
3	1127.00	-53
4	516.00	-24
5	198.00	-9.32
6	3423.00	-161
7	1581.00	-74.5
8	1174.00	-55
9	475.00	-22.4
10	249.00	-11.7
11	3178.00	-150
12	2007.00	-95
13	2979.00	-140
14	2332.00	-110
15	2799.00	-132
16	2601.00	-122
17	2954.00	-139
18	2798.00	-132
19	3532.00	-166
20	1251.00	-58.9
21	280.00	-13.2
22	3472.00	-164
23	2974.00	-140
24	2605.00	-123

The dies at 90°C, even if the activity is only 5% of the original activity, CDTA is present (ie free Mg<sup>2+</sup>) Therefore, must kill DNase I at The dies a little at = w/ CDTA, maybe 10% loss of activity.

$$140 \text{ pmole} \left( \frac{23.1}{2} \right) / 10,000 \times 3 = 0.49 \mu\text{M} \text{ (expected 0.}$$

A no killing  
A 90° 5'  
A 90° 10'  
A 90° 30'  
A 90° 1hr  
B no killing  
B 90° 5'  
B 90° 10'  
B 90° 30'  
B 90° 1hr  
A 75° 30'  
A 75° 1hr  
A 75° 2hr  
A 75° 4hr  
B 75° 30'  
B 75° 1hr  
B 75° 2hr  
B 75° 4hr  
Tie, no heat or CDTA  
Tie, 90° 10'  
Tie, 90° 1hr  
Tie, 75° 1hr  
Tie, 75° 4hr

A = 50 units Tne : 5 units DNase I  
B = 50 units Tne : 1 units DNase I

Nicking assay - after treat to kill DNase I, the rxn incubated w/ 1ug  $\phi$ X174 for 3hr at 23°C. to rx any remaining DNase I supercoiled

note that no untreated DNA was run as a pool control for how 1ug looks the Tne lanes serve as t control because Lig<sup>fl</sup> show Tne has no endonuclease

The 75°C 4hr treatment CDTA and the lower level DNase was best for kill the low level of DNase I degrades 1ug easily

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Dat

8/17/95  
cc  
Polamp

8

21/95

Recorded by

Dawn P. Pohl

8/17/95

T Page 1

Repeat assay of ... with -20°C, 4°C side by side

g N	Expt#		SAM	CPM1	ave	-20/4C
4°C	1-3	(2 planned)				
20°C	4-6					
4°C	7-9					
20°C	10-12					
4°C	13-15					
20°C	16-18					
4°C	19-21					
-20°C	22-24					
Vase	25					
Nase	26					
mock	27					
mock	28					
Nase	29					
Nase	30					
mock	31					
mock	32					
1/4 stock	33					
1/4 stock	34					
1/4 stock	35					
1/4 stock	36					
1/4 stock	37					
1/4 stock	38					
ions:						
and 4hr Tne treated with						
set and mock rxns:						
2ul eng, 0.47 u/wl P. 175						
45ul Tag dil buffer						
47ul of 0.02 u/wl						
1/4 Tne stock was diluted						
0.47 u/wl and then to						
2 u/wl → 2ul 36 u/wl stock of Tne 5-7-95						
+ 12.4ul Tag dil buffer						
14.4ul of 5 u/wl						
2ul of 5 u/wl + 19.28ul Tag dil buffer / 21.28ul of .47 u/wl, then diluted						

1E	1	7816.00	8440	90%
4E	2	9112.00		
	3	8393.00		
	4	7582.00		
20	5	7182.00	7558	
	6	7910.00		
	7	6265.00		
	8	5771.00		
4E	9	6916.00	6317	102%
	10	6005.00		
	11	6410.00		
	12	6913.00		
7E	13	7478.00	7711	96%
	14	7917.00		
	15	7738.00		
	16	7016.00		
11E	17	7461.00	7385	
	18	7679.00		
	19	5526.00		
	20	5095.00		
	21	5012.00	5211	98%
	22	5396.00		
	23	5050.00		
	24	4855.00		

30' Dna [25 2887.00] 2835 are 70%  
 30' Mock [26 2783.00] 2814 70% 5 u/wl stock dilution  
 4 hr Dna [27 2672.00]  
 4 hr Mock [28 2956.00]  
 36 u/wl [29 3095.00]  
 5 u/wl [30 3280.00]  
 31 2833.00  
 32 3113.00  
 33 4234.00  
 34 3968.00  
 35 4606.00  
 36 4839.00  
 37 3683.00  
 38 4399.00  
 39 472.00  
 40 138228.00

4041x  
 12-2835 = 70% 12000  
 4041  
 after 30' Dna treatment  
 at 75°C  
 2ul eng.  
 45ul Tag

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Recorded by		To Page No.	

Fig No. \_\_\_\_\_

treatment of Tne with DNase I: 2 rxns of

87.6  $\mu$ l H<sub>2</sub>O

10  $\mu$ l 10x DNase I buffer ~~CF=0.01~~

1.39  $\mu$ l Tne, 36  $\mu$ l, 5-7-95 prep

1  $\mu$ l DNase I, 10  $\mu$ l

100  $\mu$ l

test in PCR  
kin filled

remove 10  $\mu$ l to 400  $\mu$ l

90  $\mu$ l 1 rxn 30 min RT

1 rxn 4 hr RT  
+ 15 min

+ 5.74  $\mu$ l 50mM EDTA CF = 3mM

new vol = 95.74  $\mu$ l CF<sub>Tne</sub> = 0.47  $\mu$ l

at 75°C for 4 hr in 9600 PCR machine  
12 AM - 4 PM, put one ice

control reactions without DNase I: 2 rxns of

88.6  $\mu$ l H<sub>2</sub>O

10  $\mu$ l 10x DNase I buffer

1.39  $\mu$ l Tne, 36  $\mu$ l, 5-7-95

100  $\mu$ l

mock reaction

remove 10  $\mu$ l from each to test in PCR

ie. no killing treatment

90  $\mu$ l 1 rxn 30 min RT

1 rxn 4 hr RT  
+ 15 min

+ 5.74  $\mu$ l 50mM EDTA CF = 0.47  $\mu$ l  
Tne

at 75°C for 4 hr in 9600  
put on ice

To Page No. \_\_\_\_\_

ed & Understood by me,

Polanco

Date

8/21/95

Invented by

Recorded by

Paulson Conf

Date

8/17/95

From Page No. \_\_\_\_\_

PCR rxns: 0.5, 1, 2, 4 units Tne  
 done 8/17/95  
 long smear conditions and conditions to make specific  
 - test DNase I treated Tne  
 - test Tne that has been through a mock DNase I treatr  
 - test fresh Tne

mix A - to make a long smear

- for 14 rxns 1151.22ul H<sub>2</sub>O

28ul 1M Tris 8.5 C<sub>f</sub> = 20mM

note: in the rxn with  
 4 units Tne, the  
 [KCl] will be 55mM

23.38ul 3M KCl C<sub>f</sub> = 50mM

29.4ul 50mM MgCl<sub>2</sub> C<sub>f</sub> = 1.05mM

28ul 10mM dNTPs C<sub>f</sub> = 200uM

1260ul

mix B - to make 380bp product

- for 14 rxns 1043.98ul H<sub>2</sub>O

28ul 1M Tris 8.5

~~39.4~~ 39.42ul 3M KCl C<sub>f</sub> = 85mM

dilution of fresh Tne

30.4ul 50mM MgCl<sub>2</sub> C<sub>f</sub> = 1.3mM

30.4ul 5-7-95 stock

28ul 10mM dNTPs C<sub>f</sub> = 200uM

2ul stock

28ul 20uM anchor primer C<sub>f</sub> = 400mM

151.2ul Tag 513

28ul 20uM 6681 primer C<sub>f</sub> = 400mM

153.2ul of 0.4pu/l

28ul 50pg/ul M13 RF C<sub>f</sub> = 100pg/rxn

mix A 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24  
 90 ————— 1

mix B 90 ————— 1

H<sub>2</sub>O 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49

DNase treated Tne 1.1 2.13 4.26 8.51 1.1 2.13 4.26 8.51

mock treated Tne 1.1 2.13 4.26 8.51 1.1 2.13 4.26 8.51

\*fresh Tne 1.1 2.13 4.26 8.51 1.1 2.13 4.26 8.51

100ul rxns started on ice.

T Page 5

Witness d &amp; Underst d by m ,

Dat

Inv nt d by

Dat

Polamp

8/21/95

R corded by

Paula Smith

8/18/95

g N .

R in Lab 15 9600 - 1 min 94°C

35  
4105  
30 sec 94°C  
30 sec 55°C annealing  
2 min 72°C elongation  
4°C

program 76  
method links 71, 75, 74

50 mM KCl  
1.05 mM MgCl<sub>2</sub>

85 mM KCl  
1.3 mM MgCl<sub>2</sub>

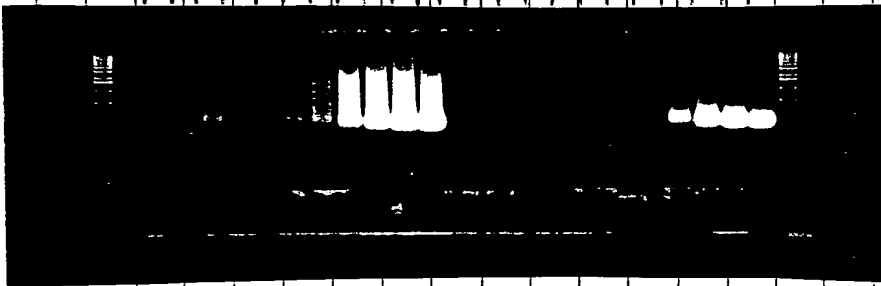
The treated w/ DNase I  
for 30' so the  
1.0 DNase I

7-95 enzyme  
units

Tne w/ DNase				mock				fresh				Tne w/ DNase				mock				fresh			
0.5	1	2	4	0.5	1	2	4	0.5	1	2	4	0.5	1	2	4	0.5	1	2	4	0.5	1	2	4

Mock rxn = Tne. without  
DNase I taken  
through all the  
DNase I treatment  
steps

Fresh = untreated  
The used directly  
from -20°C stock



ste: 85 mM KCl did not prevent the small smear from forming

To Page No. \_\_\_\_\_

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Cawson Comb

Date

8/18/95

From Page No. \_\_\_\_\_

follow P 138, 9

lot CK041

lot EJP41

no  
enzyme

①

②

③

2.1

Roxing (B&amp;D system)

147.5  $\mu$ l147.5  $\mu$ l19.67  $\mu$ l

= 0.1

0.2

1750

7

\* see P. 75

Klenow exo(-)

lot

CK041 P63, 7541  
dil in Tag SB to 4  $\mu$ l

2.5

20

EJP41 P63, 13041  
dil in Tag SB to 4  $\mu$ l150  $\mu$ l

2.5

150  $\mu$ l0.33  $\mu$ l Tag SB  
20  $\mu$ l  
2.2  $\mu$ l BJ

41°C in 9:00

remove 20  $\mu$ l to 2.2  $\mu$ l 10xBJ + 100 mM EDTA  
at 30, 1, 2, 5, 30, 90, 2 hr

start 1:2

well#

2-7

8-13

load on 2% Agarose

③

①

②

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Date

8-21-95

T Pag



Experiments on DNase I treated Tne  
Mg<sup>2+</sup> titration & mixing expt.

g N \_\_\_\_\_

crase. To determine if & how Tne was damaged by the DNase I treatment. p. 178 back rxns show low polymerase activity in the PCR, so the 75°C treatment w/ EDTA affected Tne even if DNase I was not present. A unit assay showed most of the activity was still present (~25% died). Today, we'll add back Mg<sup>2+</sup> in case there was more free EDTA than we thought. We'll also try poisoning a fresh Tne rxn w/ the treated Tne.

Mg<sup>2+</sup> titration 1.05, 1.3, 2 mM MgOAc  
smear & product conditions  
1 unit DNase treated Tne (30' treatment)  
~~1 unit fresh Tne~~  
1 unit fresh Tne from 5<sup>u</sup>/ul stock

ar mix for 12 rxn → 24ul 1M Tricine pH 9 Cf = 20mM  
30ul 2M KOAc Cf = 50mM  
24ul 10mM dNTPs Cf = 200uM  
882ul H<sub>2</sub>O  
960ul

↓ [B]  
280ul mix  
14.7ul 25mM MgOAc  
Cf = 1.05mM  
20.3ul H<sub>2</sub>O  
use 90ul/rxn

↓ [C]  
280ul mix  
18.2ul 25mM MgOAc  
16.8ul H<sub>2</sub>O  
Cf = 1.3mM

↓ [D]  
280ul mix  
+ 28ul 25mM MgOAc  
7ul H<sub>2</sub>O  
Cf = 2mM

ul mix<sup>B</sup> + 3 ul Tne treated w/ DNase 30' + 7ul H<sub>2</sub>O = 100ul \* Tne at 0.33<sup>u</sup>/ul, as determined by unit assay p. 73 NB 10 & p. 180 NB 11  
1 mix<sup>B</sup> + 3ul Fresh Tne<sup>Δ</sup> + 7ul H<sub>2</sub>O Δ diluted 5<sup>u</sup>/ul stock to 0.33<sup>u</sup>/ul  
5ul of 5<sup>u</sup>/ul Tne 5-7-95  
70.8ul of Taq SB  
75.8ul of 0.33<sup>u</sup>/ul Fresh Tne  
ul mix C + 3ul treated Tne + 7ul H<sub>2</sub>O  
ul mix C + 3ul Fresh Tne + 7ul H<sub>2</sub>O  
ul mix D + 3ul treated Tne + 7ul H<sub>2</sub>O  
ul mix D + 3ul Fresh Tne + 7ul H<sub>2</sub>O

To Page No. 180

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Polanco

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8/21/95

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R corded by

Date

8/21/95

From Page No. \_\_\_\_\_

SAP CPMI

1	2952.00
2	2739.00
3	4871.00
4	3459.00

2846

4115

68%

}

}

The (ONase treated 30') dil  $\frac{1}{23.5}$ 

Lig the stock 5% 5-7-15  
 diluted to 0.47 u/l (same as ONase)  
 treated above and then diluted  
 $\frac{1}{23.5}$

unit assay <sup>same</sup> as P73, 10

conclude The lost ~70% activity from  
 killing 4 hr killing of ONase I at 75°C (P175)

product mix for  $Mg^{2+}$  titration:for 12, 100ul rxns = 24ul 1M Tricine pH 9  $C_f = 20mM$ 51ul 2M KOAC  $C_f = 85mM$ 24ul 10mM dNTPs  $C_f = 200uM$ 24ul 20uM 6681 primer  $C_f = 400nM$ 24ul 20uM anchor primer  $C_f = 400nM$ 24ul M13RF, 50pg/ul  $C_f = 100pg/rxn$ 789ul  $H_2O$ 

960ul

Ⓔ

for 3.5 rxns

280ul mix

14.7ul 25mM  $MgCl_2$ 20.3ul  $H_2O$   $C_f = 1.05mM$ 

315ul

use 90ul/rxn

Ⓕ

280ul mix

+ 18.2ul 25mM  $MgCl_2$ 16.8ul  $H_2O$ 

315ul

 $C_f = 1.3mM$ 

Ⓖ

280ul mix

+ 28ul 25mM  $MgCl_2$ + 7ul  $H_2O$ 

315ul

 $C_f = 2mM$ 

T Page No

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J. Polak

Date

8/28/95

Initiated by

R. Cord d by

Date

8/22/95

ag N \_\_\_\_\_

reactions → ~~DT~~ = Tne. treated w/ DNase for 30' p. 175, 1 unit, 0.33%  
 FT = Fresh untreated Tne diluted to 0.33%<sup>10</sup>, 1 unit

smear condition  
 50 mM KOAc  
 20 mM Tricine pH 9.0  
 no template no primers

product condition =  
 85 mM KOAc  
 20 mM Tricine pH 9  
 anchor primer + 6681 primer on m13

[KOAc] mM	1.05		1.3		2 mM		1.05		1.3		2	
zyme	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT
1 unit of eng./rxn												
0.8	1	3	4	6	7	9	10	12	13	15	16	18
20ul of each 100ul rxn was run on a 0.8% gel p. 18.4 →												

We had also planned to do rxns with 4 units of the DNase I treated Tne, but there was not enough of the eng. to set up these rxns

~~Mg<sup>2+</sup> was omitted from rxn by mistake~~  
 being experiment to determine if the DNase I-treated Tne has a "poisonous" substance in it - mix untreated Tne w/ DNase-treated Tne. smear & product conditions 1 u untreated + 0 treated

mix for smear, 6 rxns = 12ul 1M Tricine pH 9 (C=20mM)  
 15ul 2M KOAc C=50mM  
 12ul 10mM dNTPs C=200uM  
 441ul H<sub>2</sub>O

480ul → use 80ul/100ul rxn

80ul mix + 3ul fresh Tne (0.33%<sup>10</sup>) + 17ul H<sub>2</sub>O  
 80ul mix + 3ul " + 1.52ul treated Tne + 15.48ul H<sub>2</sub>O  
 80ul mix + 3ul " + 3ul " + 14ul H<sub>2</sub>O  
 80ul mix + 3ul " + 6ul " + 11ul H<sub>2</sub>O  
 80ul mix + 3ul " + 12ul " + 5ul H<sub>2</sub>O

To Page No. \_\_\_\_\_

is d &amp; Understood by me,

Polansky

Date

8/28/95

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Recorded by

Carolyn Lamb

Date

8/21/95

From Page No. 181

2<sup>nd</sup> try at the mixing expt. - I forgot to add MgOAc to the rxns on

smear buffer: C<sub>f</sub> = 20mM Tricine pH 9.0  
 50mM KOAc  
 1.05mM MgOAc  
 200μM dNTPs

no primers or template  
 mix 1 unit of fresh Tne w/  
 0, 0.5, 1, 2, 4 units of  
 mock-treated Tne. p. 17:

for 6 rxns = 12ul 1M Tricine pH 9 ✓  
 15ul 2M KOAc ✓  
 25.2ul 25mM MgOAc ✓  
 12ul 10mM dNTPs ✓  
 41.5.8ul H<sub>2</sub>O ✓

480ul → use 80ul mix per 1, 100ul rxn

product buffer: C<sub>f</sub> = 20mM Tricine pH 9  
 85mM KOAc

1.3mM MgOAc  
 200μM dNTPs  
 400nM 6681 primer  
 400nM anchor primer  
 100pg/1rxn M13mp19 RF template

mix 1 unit fresh Tne w/  
 0, 0.5, 1, 2, 4 unit  
 of mock treated Tne  
 p. 17

A unit assay was done on the <sup>mock</sup> treated Tne on Fri 8/18/95 p. 75 N<sub>1</sub>  
 We'll assume a concentration of 0.33<sup>mock</sup> ul, which was the an  
 concentration on 8/18. The base treated Tne had not lost any more acti  
 at 4°C over 3 days, as shown by unit assay on 8/21/95 p. 180. It's likely  
 the mock didn't loose an

for 6 rxns = 12ul 1M Tricine pH 9 ✓

25.5ul 2M KOAc ✓

31.2ul 25mM MgOAc ✓

12ul 10mM dNTPs ✓

12ul 20μM 6681 ✓

12ul 20μM anchor ✓

12ul 50pg/ul M13 RF in TE ✓

363.3ul H<sub>2</sub>O ✓

480ul, use 80ul mix / 100ul rxn

T Pag N

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R cord d by

Date

8/22/95

Page No. _____	19	20	21	22	23	24	25	26	27	28	29
1st mix /	80ul										
2nd mix /						80ul					
Tne /	3ul (unit)										
2nd-treated 0.33%ul	0	1.52	3	6	12	0	1.52	3	6	12	
5											
7	17	15.48	14	11	5	17	15.48	14	11	5	
	100ul										

94°C 1 min

94°C 30 sec

55°C 30 sec

72°C 2 min

4°C - hold

Method 76, Lab 15 9600 Method 103 Lab 16 9600 35 cycles

Dilution of fresh Tne : 5ul of 5% Tne 5-7-95 Lys stock  
70.8ul Tne SIB

75.8ul of 0.33%ul fresh Tne

8% TAE gel w/ EtBr, 20ul of each rxn was run on gel

To Page No. \_\_\_\_\_

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8/28/95

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Book No. \_\_\_\_\_

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From Page No. \_\_\_\_\_

Results:

$Mg^{2+}$  titration  
 p. 179  
 DT = Tne treated 30' with DNase I p. 175  
 FT = fresh, untreated Tne  
 smear cond. 50 mM KOAc  
 no template/primer  
 product cond. 85 mM KOAc

Mixing expt.

p. 182

1 unit Fresh Tne

+ increasing units of mock treated

Tne (no DNase)

smear cond. product cond.

units of mock treated

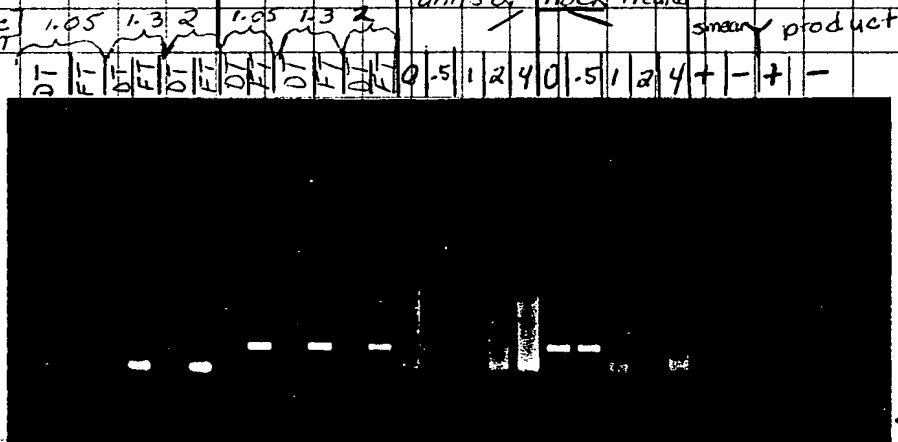
Rnase treatment of Tne

p. 185

+ = Tne treated w/ RNase

- = Tne not treated w/ RNase

1 unit eng →


 cse.  
 8/23/95
Conclusions from  $Mg^{2+}$  titration expt:

A unit assay was done on the DNase I treated Tne (see p. 180) and Fresh Tne. Equal units of the DNase I treated and fresh Tne were used in the  $Mg^{2+}$  titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the 75°C incubation with EDTA (to kill the DNase I) damaged Tne. Therefore we do not yet know if treating Tne w/ DNase I can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNase I activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our nicking assay p. 174 shows that 1 µg of p174 was not degraded in 3 hr and was only nicked a little (~10%) at the DNase I killing treatment

2) in a previous expt (p. 177) the mock-treated Tne showed the same low activity in a PCR as the DNase I treated Tne. So, the DNase was not responsible for the low amount of smear products made.

Tne + EDTA died at 94°C. maybe adding more may overcome the damage to Tne. p. 174

Further expts to try: measure thermostability of the 75°C w/ EDTA treated Tne, by cycling the Tne to 94°C before doing the unit assay → purify the DNase I-treated Tne away from DNase or "poison"-gel

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Date

8/28/95

Inventor by

Recorded by

Dariusz Pami

Date

8/23/95

To Page No.

Expt.: RNase A + 1 + treatment of Tne. Is RNA the bad seed that primes the smear.

Pr j ct No. \_\_\_\_\_

185

PCR 1 is 1+2

PCR 3 is 5+6

See result on p. 184

PCR 2 is 3+4

PCR 4 is 7+7

pt 3 → RNase

#1, ~~2~~

small worth of buffer w/ 100ul worth of dNTP, target, primers

smear V V 1ul Tricine

V V 1.25ul 2m KOAc

V V 2.1 ul 25mm MgOAc

V V 2 ul 10mm dNTP

V V 2ul 6681 20m

V V 2ul anchor

V V 2ul m13, 50pgul

1 RNase T1 } both diluted 10 fold  
1 RNase A } in 10 mM Tricine

35.65 ul H<sub>2</sub>O

50ul

T1 1460u/ul

A 10mg/mL = 10ug/ul

1 ul → 40ug RNA/mL

dil 10x  
use  
1ul

car Tne V V

V V

V V

int solid →

1ul Tricine #2

1.25ul 2m KOAc

2.1ul 25mm MgOAc

2ul Tne

Li25uA

want 1 unit  
→ dil to 0.5u/1 in SB

1 ul RNase I

1 ul RNase A } dil 10x in 10mm Tricine

ul H<sub>2</sub>O

46.65ul

50

15' 37°C → mix → PCR

same w/o RNase

#3, 4

25C  
8/22/95

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J Polansky

Date

8/28/95

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Recorded by

Paula Combs

Date

8/22/95

From Page No. \_\_\_\_\_

expt 3 RNase:

for prot<sup>+</sup>

1ul Tricine 1M

✓ ✓ 2.13ul 2M KOAc

✓ ✓ 2.6 ul 25mM MgOAc

✓ ✓ 2ul dNTP 10mM

✓ ✓ 2ul 6681 20mM

✓ ✓ 2ul anchor 20mM

✓ ✓ 2ul m13 50pg/ul

1 RNase

1 RNase

✓ ✓ 34.27 H<sub>2</sub>O

50ul

✓ ✓ 1 ul Tricine

✓ ✓ 2.13 2M KOAc

✓ ✓ 2.6 25mM MgOAc

2 ul Tric 0.5u/l

✓ ✓ mix 40.27 H<sub>2</sub>O

1 RNase

~~it~~✓ <sup>50</sup> same w/o RNase

#5

8/22/95  
CS

#6

#7

#8

T Pag 1

With ss d &amp; Und rstood by me,

Dat

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Inv nt d by

Record d by

Lawton Paul

Dat

8/22/95



g N 184

conclusions from the mixing expt. on p. 184:

The purpose of the mixing expt was to see if 0.5, 1, 2, 4 units of mock treated Tne could poison a PCR with 1 unit of fresh Tne. The mock treated Tne received the 4hr 75°C EDTA treatment but did not contain any DNase I.

The mock-treated Tne did not poison the ability of fresh Tne to make a <sup>10<sup>10</sup>ng</sup> smear under the standard smear buffer conditions of 50mM KClAc, 20mM Tricine, 4.05mM MgOAc. The differences in smear intensity are probably just representative of <sup>typical</sup> variation in smear intensity. Therefore the mock-treated Tne does not contain a poison that is effective at the levels tested.

The mock-treated Tne also did not poison fresh Tne's ability to make a specific product (0.5u mock w/ 1u fresh still made product). The smears seen with 1, 2, 4u mock probably are the result from having too many total units. Using more than 1 unit Tne/100ul rxn always results in a smear. The amt of mock was not enough to make a ~~smear~~ 1 unit mock may not be exactly the same as 1 unit of treated (which doesn't show any PCR activity in the Mg<sup>2+</sup> titration expt) and the window for ~~too~~ activity is probably very narrow. - More controls & expt should be done to confirm the absence of a poison.

conclusions from RNase expt - Tne treated with RNase A + RNase T<sub>1</sub> and then used directly in a PCR. If RNA is the "bad seed" RNase might cure the formation of a smear and increase product yield. The RNase treatment had no effect on either smear formation or product yield. We conclude that RNA is not priming the smear reaction.

To Page No. \_\_\_\_\_

ed &amp; Understood by me,

Polansky

Date

8/28/95

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Recorded by

Cecilia Fombi

Date

8/22/95

**From Page No.\_\_\_\_**

Mg<sup>2+</sup> titration  
DT = Time treated 30' with  
NaOH T = 135

<p>F.T = fresh, untreated Tne smear cond.          50mm KOAc          no template/primer</p>	<p>✓ product cond.          85mm KOAc</p>
--	---

Mixing expt.  
p. 182

Unit Fresh Tne  
+ increasing units  
of mark treated

Smear cond	product cond
units of	rock treated

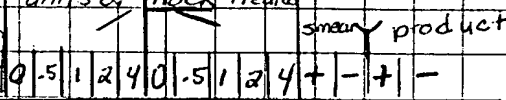
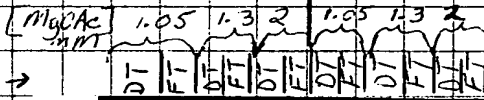
### Rnase treatment of Tne

p. 185

† = Tne treated w/ RNase

- = The not treated w/ RNase

1 unit enz  $\rightarrow$



smear product

CSL  
8/23/95

Conclusions from  $Mg^{2+}$  titration expt:

A unit assay was done on the DNase I treated Tne (see p 180) a. Fresh Tne. Equal units of the DNase I treated and fresh Tne were used in the  $Mg^{2+}$  titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the  $75^{\circ}C$  incubation with EDTA (to kill the DNase I) damage Tne. Therefore we do not yet know if treating Tne w/ DNase can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNase I activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our nicking assay p. 174 shows that 1  $\mu$ g of  $\phi$ X174 was not degraded in 3 hr and was only nicked a little (~10%) after the DNase I killing treatment. 2) in a previous expt (p. 177) the mock-treated Tne sh. the same low activity in a PCR as the DNase I treated Tne. So, the DNase was not responsible for the low amount of smear products made.

$\text{In}^+ \text{GTA}$  died  
 at 9:40 maybe  
 adding more  
 comp. will become the  
 damage to  $\text{In}^2$   
 p. 174

Further expts to try: → measure thermostability of the 75°C w/ CDTA treated Tne, by cycling the Tne to 94°C before doing the unit assay  
→ purify the Dnaase I-treated Tne away from Dnaase or "poison"-del

**Witnessed & Underst d by m .**

**Dat**

**Invent d by**

## Dat

Recorded by

8/23/95

To Page No. 11

ag N \_\_\_\_\_

2.2 X reactions,  $V_p = 0.1$  each

Rx mix (B&D)

Cf at 1X

$\text{H}_2\text{PO}_4$  pH 7.6  
P61

~~33~~  $\mu\text{l}$

16.5  $\mu\text{l}$  ✓

50 mM

2 NTPs 10 mM

~~13.2~~  $\mu\text{l}$

6.6 ✓

200  $\mu\text{M}$  each

BSA nuclease free

~~4.4~~  $\mu\text{l}$

2.2 ✓

0.1 mg/ $\mu\text{l}$

celysated *Stratagene* cat 50004157

50% glycerol

~~180.8~~  $\mu\text{l}$

~~90.4~~ 86.9 ✓

13.17% (includes contributed by *Klenow* ex.)

1 M Mg  $\text{Cl}_2$

~~4.6~~

2.31 ✓

7 mM

2 must go in after

204 is diluted to

1.0 ppt of Mg  $\text{PO}_4$ )

33 correct. mp 19

~~52.8~~  $\mu\text{l}$

26.4 ✓

1389: 41/50  $\mu\text{l}$  rxn vol

0.12 pmol primer

0.24 pmol *circ*

circ primer = 2

note 5  $\mu\text{l}$  K<sub>16</sub>

295  $\mu\text{l}$  rxn

contributes

$(\frac{5}{300})$  50% glycerol

= 0.833%

to Cf

∴ overall C

is 14% glycerol

in Rxn

H<sub>2</sub>O

~~360.14~~

183.6 ✓

~~180.1~~

$V_p = \frac{649}{324.5}$

To Page No. \_\_\_\_\_

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*J. B. Jones*

Date

8/28/95

Invented by

*F. C. et*

Date

7-28-95

Recorded by

From Page No. \_\_\_\_\_

mix A

for 4 Rxns (Cf 100)

10X PCR buffer  
50 mM MgCl<sub>2</sub>✓ 40  
✓ 56(Cf = 1100 CPM/pmol &  
G 200  $\mu\text{M}$  each)H<sub>2</sub>O✓ 260  
356use 95  $\mu\text{L}$  / 100  $\mu\text{L}$  Rxn

(1) (2) (3)

m A

89  $\mu\text{L}$ 

→

✓ (53.6 u)

The. Ciz 36  $\mu\text{L}$ 

2.6

✓

The P L 7-22-95

19  $\mu\text{L}$  (see P 127, 11)

5

✓

95 u

rTos EKBTI

dil to 36  $\mu\text{L}$ 

2.6

✓

93.6

Try SB

2.4

2.4

✓

heat reactions 1' 94  
then lower heat to 7  
then start reaction

dCTP, dNTP mix\*

6

✓ 100  $\mu\text{L}$  →by addition  
of dCTP, dNTP  
mix

X mix

 $^{32}\text{P}$  dCTP 16  
100  $\mu\text{L}$  eachremove 10  $\mu\text{L}$  to 5  $\mu\text{L}$  regl stop solution  
at 1 2 5 15 30 60 90 min10 min JNT P<sub>2</sub> 8  
v.f. = 64

run of 7% PAGE

with dDA, dDT and PFI (Reactions #22, 23, 24  
at top of P7P) reloaded here as  
number 22, 23, 24 also. 23 is no Eng

T Page 8

Witnessed &amp; Understood by me,

S. Polans

Dat

8/28/95

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Record d by

Dat

8-28-95

86

see P155-157 within

Project No.

Book No.

TITLE

Provisionality

The FY

vs 1

vs Althman

From Page No. 60811

Mix A process

(for 37 Rxns, 40  $\mu$ l/5  $\mu$ l R

from P.75

32 P33 run correct. mp19

✓ ✓

130  $\mu$ l

7.8 pmol circles

circle/prime = 2

3.9 pmol prime

0.06 pmol circle

H<sub>2</sub>O

✓ ✓

1109.5  $\mu$ l

for 37 Rxns

10 x PCR buffer

✓ ✓

147 (1x at 40  $\mu$ l)use 40  $\mu$ l/Rxn50 mM MgCl<sub>2</sub>

✓ ✓

55.5  $\mu$ l (1x at 50  $\mu$ l/Rxn)

Cp = 1.5 mM Mg

10 mM DTPS

✓ ✓

37 (1x at 50  $\mu$ l)1x at 50  $\mu$ l/Rxn = 2

$\mu$ l = 1.47  $\mu$ l  
 3.84 x The Liz well H  
 The FY  
 5.4  $\mu$ l  
 (Altman  
 5.4  $\mu$ l)

sol dil	.00005 $\mu$ l
	.0001
	.0002
	.0004
	.0008
	.0016
	.0032
	.0064
	.0128
	.0256
	.0512

#1

12

23

2

13

24

3

14

25

4

15

26

5

16

27

6

17

28

7

18

29

8

19

30

9

20

31

10

21

32

11

22

33

for 40  $\mu$ l mix

= 0.21 pmol

50  $\mu$ l

n same as

put 2  $\mu$ l pol into 8  $\mu$ l of 1.25 x PCR buffer  
 preheat to 70°C 1 min in 9600

start with 40  $\mu$ l of Mix A process (also preheated to 70  
 stop at 2 min with 25  $\mu$ l cycle seq stop sol

number "0" is Mix A process 40  $\mu$ l  
 Tag SB 2  
 H<sub>2</sub>O 8  
 cycle seq stop 50  $\mu$ l  
 25  $\mu$ l

To Page 1

Witnessed &amp; Understood by m

Dat

Invent d by

Dat

a/7/95

R c rded by

8-87-95

From Page No. \_\_\_\_\_

Fidel Pri (P54)  
100  $\mu$ M  
(its a 27mer)5  $\mu$ l ✓

(500 pmol total)

 $\gamma$ -<sup>32</sup>P ATP 10 mCi/ml  
(3.33  $\mu$ M)

10 ✓

(33 pmol total)

5X Knaal buffer  
PNK 10<sup>4</sup> U  
H<sub>2</sub>O

10 ✓

24 ✓

50  $\mu$ l

37°C, 30' → 70°C, 5'

[dT]

25  
5

[dA]

25  
5

✓

Fidel Temp (dT)

Fidel Temp dA

P54 100  $\mu$ M

10 mM Tris pH 8

242.78

277.8

-  $\mu$ l ✓-  $\mu$ l

vf

500

(1000 pmol)  
( $\frac{500 \text{ Temp}}{25 \text{ pri}} = 2$ )

Cf = 900 nM primer

↓

90°C 2 min

↓

cool slow

use 5  $\mu$ l / 50  $\mu$ l extension reaction  
for Cf = 90 nM primer

T Pag Nc

Witness d &amp; Understood by me,

Date

9/7/95

Invent d by

Rec rd d by

Dat

8-31-95



PCR with OAPDH, gloom, Chery vs PCR buffer  
 for The WT and ΔSFY  
 see P 78, 11 for wt conditions

Proj # No. \_\_\_\_\_

B k No. \_\_\_\_\_

93

Tag No. \_\_\_\_\_

94°C 1 min

94°C 30 s

55°C 30 s

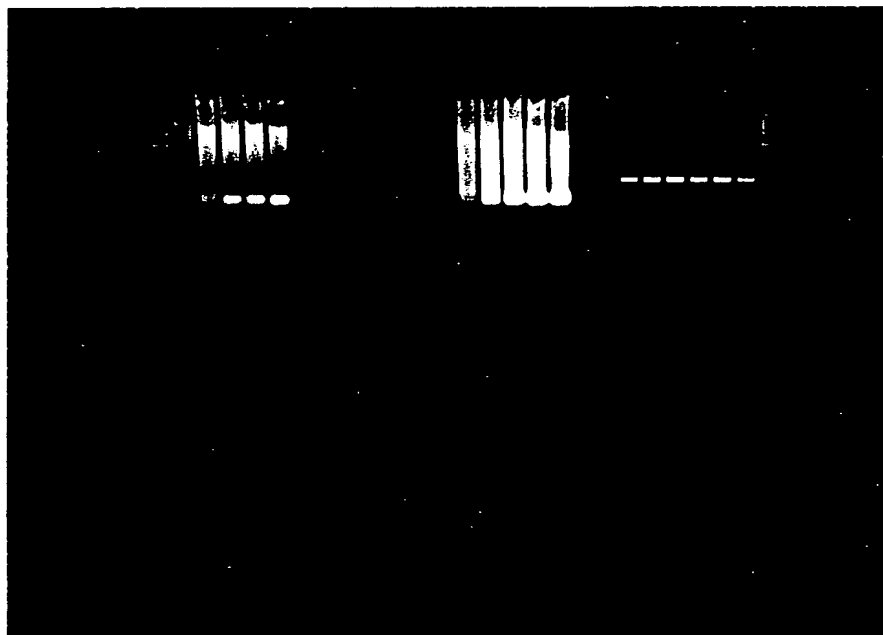
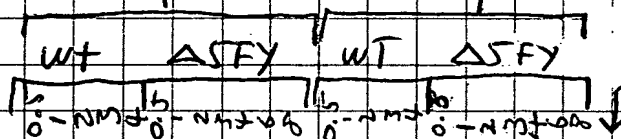
72°C 2 min

} 35 cycles

4°C hold

(set BS+EDTA added ≤ 30' after finish  
 Chery buffer Tag PCR buffer  
 2 units rTag pol in Tag PCR buffer

The units



← 267

Result: only get expected 267 bp product for The ΔSFY if  
 PCR buffer used consistent with lower ionic strength  
 of PCR buffer helping the less processive deleted pol  
 (Chery is 55 mM KOAC compared to 10 mM)

To Page No. \_\_\_\_\_

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S. Polansky

9/2/95

Recorded by

7-1-95



Project

Book No.

TITLE

PCR with Tne Δ5 FY

94

From Page No.

Mix A

10x PCR buffer  
 5x Thermo + dNTPs  
 50 mM MgCl<sub>2</sub>  
 Human DNA 20 ng/μl  
 H<sub>2</sub>O  
 Tne Δ5 FY

100 μl  
 200 μl  
 21 μl  
 20 μl  
 810.5 μl  
 8.5 μl  
 980 μl

✓ for 20 PCR  
 ✓  
 ✓  
 ✓  
 ✓  
 ✓  
 ✓

(1.05 min)

	1	2	3	4	5	6	7	8	9
mix A	48 μl								
2.67 bp	1								
BDNF		1							
1.366 kb primers			1						
2.0				1					
2.82					1				
4.1						1			
5.5							1		
6.166								1	
7.5									1

94°C, 1'

94°C 30S

15°C 30S

72°C

40°C

35 cycles

start 11:35

2 min (#1-9) or 7 min (#10-18)  
 (get BJ + EDTA in ≤ 30' after finish  
 in case 3' ext is a problem)

start 7:40  
 for 7 min along need ~ 5 hrs  
 done ~ 2:30  
 need ~ 2 1/2 hr for 2 min  
 (done ~ 11:10)

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G. O. O. O. O.

Dat

9/2/95

Invent d by

R c rded by

Dat

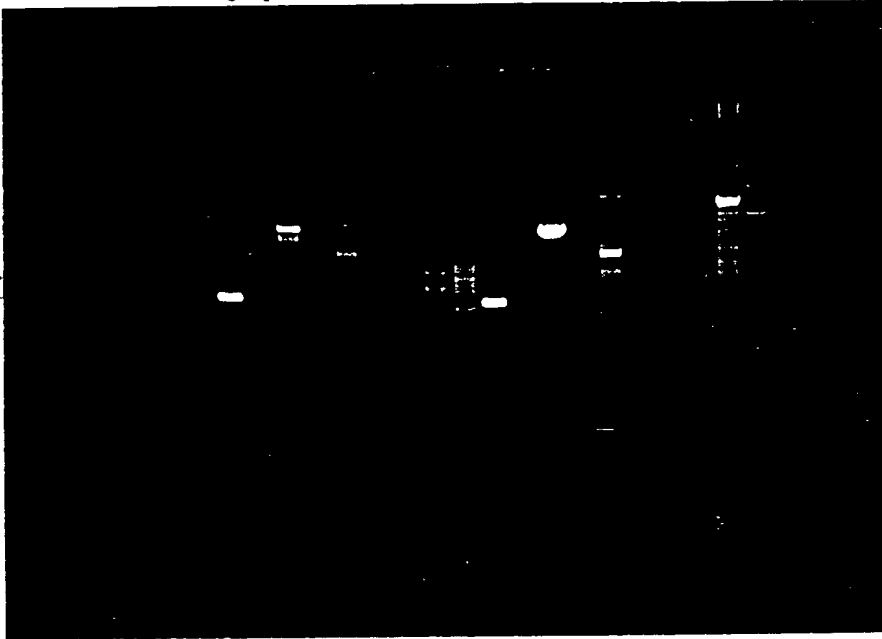
9-6-95

To Pag No

Page N \_\_\_\_\_

2' elongation 7' elongation

267 200 200 4.1 5.5 6.1 7.1



267	+	
200	+	( for 7 min along
1.36	+	
2	+	( for 7 min
2.8	-	
4.1	-	
5.5	-	
6.1	-	
7.1	-	

To Page No. \_\_\_\_\_

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9-8-95

From Page No. \_\_\_\_\_

10x Tag PCR buffer = 200 mM Tris 8.4, 500 mM KCl

10x ultimate buffer is 100 mM Tris pH 8.8, 10 mM KCl

I will try 0 10 25 50 mM KCl CF  
 (for now I'll stay with 20 mM CF Tris 8.8)

(A)

1 M Tris pH 8.8	20 $\mu$ l	✓	(CF=20 mM)	(for 10x 10)
50 mM MgCl <sub>2</sub>	21 $\mu$ l	✓	(CF=1.05 mM)	
4 dNTPs	25	✓	(250 $\mu$ M each)	
Human DNA 20 ng/l	5 $\mu$ l			
9.4 $\mu$ l Tris 0.5 M	8.5 $\mu$ l	✓	(44/50 $\mu$ l as per P)	
H <sub>2</sub> O	86.5 $\mu$ l	✓	(24/100 $\mu$ l PCR)	
	940			
				use 94 $\mu$ l / 100 $\mu$ l P

(A)

① ② ③ ④ ⑤ ⑥ ⑦ ⑧  
 94  $\mu$ l —————→

KCl

200 mM  
 500 mM  
 1 M

—	5	5	5	5	5	5	5	✓	CF= 1
								✓	2
								✓	3

primers

267 bp 16/12 h/g 1 —————→

1.366 kb  
 H<sub>2</sub>O

5  
 100  $\mu$ l —————→

cycle as per P 94 with 2 min elongation

start 7:40 AM done

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S. Olamp

Date

9/7/95

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Date

9-7-95

T Page

e N . \_\_\_\_\_

Result

note 267 bp product does  
just begin to appear  
at 5 stages and  
highest No. 4

cell of 50 mM  
concluded:

- 1) higher NaCl  
help in contrast  
to expectation that  
it would inhibit  
this distributive  
form of Tse.  
2) target DNA  
is limiting here  
since higher yields  
were obtained for  
4x more DNA in  
P 93 and 94

apparently need  $\geq 1 \mu\text{L}$  of H/S DNA (100 ng) / 50  $\mu\text{L}$  Rxn  
need only 0.25  $\mu\text{L}$  for

To Pag No. \_\_\_\_\_

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Date

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Sandra B. King

9/7/95

9-7-95

Exhibit L-176

Project Appl. No. 09/558,421

98

Book No. \_\_\_\_\_

TITLE

Prepare Tth sol. for shipping  
to Roache

From Page No. \_\_\_\_\_

They want 10,000 units and some SB as LTI expect  
include 100 mM KCl

Therefore

Tth (formerly thought to be TF1)  
4-30-95 (see PR for units)  
4.33 u/ $\mu$ l

2.5 ml

(10825 un  
total)\* 2 M KCl in 50% glycerol  
and 20 mM Tris pH 8

0.132 ml

 $V_f = 2.632$  mlfinal units = 4.1 units/ $\mu$ l\* KCl  
glycerol  
H<sub>2</sub>O  
1 M Tris pH 8

0.298 g

1 ml

to 2 ml

20  $\mu$ l $V_f =$ 

2 ml

(MW = 74.55)

To Page No. \_\_\_\_\_

Witnessed &amp; Understood by me,

Date

Invented by

Date

D. Robert Polansky

9/9/95

Recorded by

7-995